MINISTRY OF EDUCATION AND TRAINING
CAN THO UNIVERSITY

School year: 2010-2012

VO DUY THANH

Effect of NPN source and mangosteen peel (*Garcinia mangostana*) on methane production in *in vitro* incubations and on growth performance of Phan Rang sheep in the Mekong delta of Vietnam

MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES
ANIMAL HUSBANDRY

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Scientific supervisors:

1- Prof. Nguyen Van Thu

2- Prof. Dr. T R Preston

AN APPROVAL OF THE SCIENTIFIC EVALUATION COMMITTEE

The thesis with the title: **"***Effect of NPN source and mangosteen peel (Garcinia mangostana) on methane production in in vitro incubations and on growth performance of Phan Rang sheep in the Mekong delta of Vietnam***"** implemented byMrVo Duy Thanh was approved by the Scientific evaluatation Committee at the Can Tho University

Committee member **Secretary**

Opponent number 1 **Opponent number 2**

Can Tho City, 01 March, 2012

Chair person

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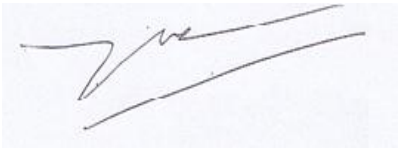

Vo Duy Thanh

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.

Author

Vo Duy Thanh

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Contents

Abstract

A series of experiments was carried out to determine the effect of: (i) calcium nitrate and mangosteen peel on methane production in an *in vitro* fermentation using molasses and cassava leaf meal as the basal substrate; (ii) potassium nitrate and mangosteen peel on methane production in an *in vitro* fermentation using molasses and *Operculina turpethum* as the basal substrate; and (iii) effects of potassium nitrate associated with mangosteen peel on growth performance and methane production in sheep.

Paper 1:

An *in vitro* incubation was used to evaluate effects of Mangosteen peel extract with calcium nitrate or urea on methane production from a substrate of molasses and cassava leaf meal. The design was a 2*2 factorial arrangement in a completely randomized block design with four replications.

Calcium nitrate as replacement for urea, and addition of Mangosteen extract, lowered methane production during the final phase (32 to 48h) of the incubation. Estimates of effects over the entire incubation period, based partially on results from similar experiments in the literature, support the effect of calcium nitrate in lowering methane production.

It was concluded that the apparently beneficial effect of calcium nitrate and Mangosteen peel extract in reducing methane production needs to be substantiated by further research.

Key words: *fermentation, gas production, tannins, saponins*

Paper 2:

The objective of this study was to evaluate the effect of the level of Mangosteen peel and potassium nitrate or urea as non-protein nitrogen source on methane production in an *in vitro* incubation. The design was a 3*4 factorial with 3 replicates. The factors were source of non protein nitrogen: urea (1.83% of substrate, DM basis) and potassium nitrate (4 or 6% of substrate, DM basis); and levels of Mangosteen peel (0, 0.5, 1 and 1.5% of substrate DM basis). The quantity of substrate was 2.5g to which were added 200ml of buffer solution and 50ml of buffalo rumen fluid taken immediately after the animal was killed in the slaughter-house. The incubation was for 48 h with measurements of gas and methane production at 6, 12, 24, 36 and 48 h. The proportion of substrate solubilized at 48h was determined by filtration, followed by measurement of ammonia-nitrogen concentration in the filtrate.

The 6% level of potassium nitrate was more effective in reducing methane production than the 4% level. Gas and methane production increased with time of incubation. Similar reductions in the above parameters were observed with increasing level of Mangosteen peel in the substrate. The ammonia concentration in the filtrate after 48h of incubation was lower when potassium nitrate was the NPN source compared with urea.

The results of this paper showed that after 48h incubation, gas and methane production, per cent substrate DM digested and methane produced per unit DM digested, were lower when potassium nitrate was the NPN source compared with urea.

Keywords: *Climate change, greenhouse gases, incubation, rumen.*

Paper 3:

Twelve female Phan Rang sheep with an initial weight of 21.3 ± 0.2 kg at 4 months of age were allocated in a 2 x 2 factorial design with 3 replicates. The first factor was non protein nitrogen source (urea or potassium nitrate); the second factor was Mangosteen peel meal at 0 or 1.5% of the diet DM.

It was concluded that feeding potassium nitrate rather than urea decreased the ratio of methane to carbon dioxide in the eructed air from the sheep. There was a tendency for methane production to be reduced by supplementation with Mangosteen peel. There was no effect of the NPN source, nor of the supplementation with Mangosteen peel, on apparent digestibility, N retention, and growth performance.

Key words: *Ammonia, digestibility, climate change, feed conversion, greenhouse gases, live weight gain, VFA*

Abbreviations

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CF	Crude fibre
CH_4	Methane
$Ca(NO_3)_2$	Calcium nitrate
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
FCR	Feed conversion ratio
KNO_3	Potassium nitrate
L	Liter
LW	Live weight
Mekarn	Mekong basin animal research network
MP	Mangosteen Peel
N	Nitrogen
NDF	Neutral detergent fibre
NH_3	Ammonia
NPN	Non-protein nitrogen
OM	Organic matter
pH	Power of/potential Hydrogen
Prob/P	Probability
SEM	Standard error of the mean
Sida-SAREC	Swedish international development cooperation agency, department for research cooperation
U	Urea

Introduction

Vietnam is tropical country with a monsoon climate located in Southeast Asia. The total area of the country is 33.2 million ha, with a population of 84 million. There are 52 million farmers, and 67% of the total labor force is working in the agricultural sector. According to Binh and Lin (2005), there are three systems of management of small ruminants in Vietnam (intensive, semi-intensive and extensive systems), whereas the two main systems for management of sheep in Phan Rang are the extensive system and the semi-extensive system (Mai et al 2003). The semi- extensive system can normally be found on large private or state farms, with herd sizes ranging from hundreds to thousands. In this system, the sheep are allowed to graze during the day time and are supplemented with feeds during the night time. In the extensive system, most sheep are privately owned and are mainly kept by rural smallholders, and the flock size is usually between 10 and 100 head. The sheep are grazing all day and brought back to the house at night time, and are not given any supplementary feed. Some exotic sheep breeds, such as Dorper and White Suffolk, were imported by the government from Australia recently for use in intensive systems in Vietnam. In these systems, the sheep are kept in individual pens and feeds are supplied entirely from the outside.

In Vietnam mangosteen is mainly distributed in the Mekong Delta with a total area of 4.9 thousand hectares and total yield of about 4.5 thousand tonnes (about 1 tonne/ha)

http://www.rauhoaquavietnam.vn). Therefore a great amount of mangosteen peel (MSP) is thrown away in the canals causing pollution of the environment. Therefore utilization of MSP for purposes such as animal production could be beneficial economically and environmentally. There have been no studies on the effect of mangosteen peel on rumen methane production in sheep. Previous studies with mangosteen peel were aimed at modifying the rumen fermentation (Suchitra Kanpukdee and Wanapat 2008). Supplementation with mangosteen peel decreasedthe acetic: propionic acid ratio and it was predicted that this would have resulted in a reduction in methane production.

Nitrate is a potent inhibitor of methanogenesis in all systems from fermentative digestion in the rumen to secondary fermentation in a wide range of systems from anaerobic biodigestors to sediments (Hungate1965; Allison et al 1981; Akunna et al 1994). In subsequent publications, Sokolowski et al (1960) suggested that nitrate in a relatively good quality diet that supported substantial growth was not apparently toxic to sheep. Tillman et al (1965) demonstrated that molybdenum had marked effects on nitrate metabolism in the rumen of sheep fed purified diets with nitrate as the sole source of fermentable N. The same author (Sokolowski et al 1969) indicated that adding 3.2% KNO_3 to a concentrate based diet, with or without added sulphur, lowered the overall growth rates of lambs offered the diet over 48d.

The hypotheses to be tested were

Experiment 1

Calcium nitrate and mangosteen peel will reduce methane production in an *in vitro* fermentation using molasses and cassava leaf meal as the basal substrate.

Experiment 2

Potassium nitrate and mangosteen peel will reduce methane production in an *in vitro* fermentation using molasses and *Operculina turpethum* as the basal substrate.

Experiment 3

There will be synergistic effects of potassium nitrate associated with mangosteen peel which will reduce methane production on sheep.

Literature review

Global greenhouse gas from ruminants

Agriculture land occupies about 40 to 50% of the Earth's land surface (IPCC 2007) and agriculture accounts for an estimated 10 to 14% of total global anthropogenic greenhouse gas emissions (IPCC, 2007). The CH_4 produced by methanogens accounts for about 25% of ruminal gases (Moate et al 1997) and it is absorbed and eructated with CO_2. Cattle produce about 150 to 420 L of CH_4/day and sheep about 25 to 55 L/day, depending on intake (Czerkawski, 1969, Holter and Young, 1992, McAllister et al, 1996).

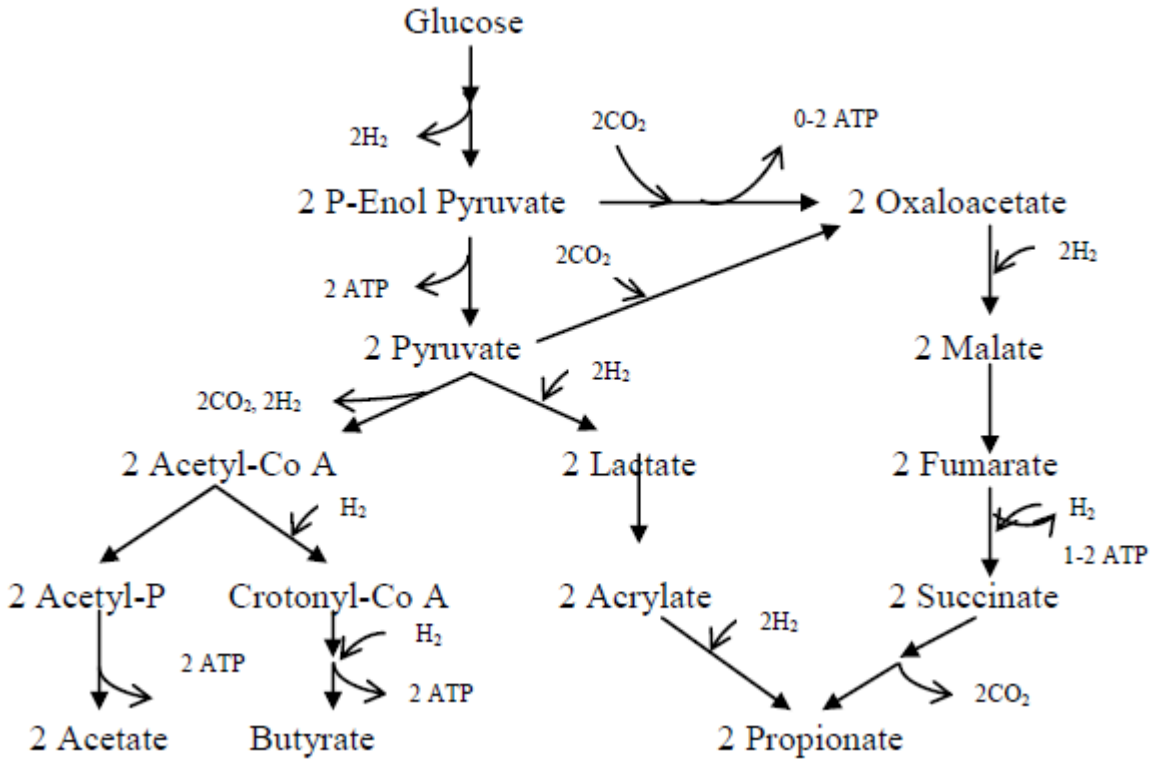

Figure 1. Fermentation pathways in the rumen. Methane is formed from carbon dioxide (CO_2) and hydrogen (H_2). (Ungerfeld and Kohn, 2006).

Livestock population in Viet nam

Small ruminants have been raised all over the country for a long time in extensive systems with low productivity. However, in recent years, more attention has been paid to small ruminant production by farmers and government. Some achievements have been made in the fields of breeding, nutrition, processingand disease prevention. Some programs and projects havebeen carried out with the support of government and international organisations that have had an impact. It is clear that small ruminant production is playing an important role in improvement of the incomes for poor farmers in the hilly and mountainous areas and is contributing to poverty and hunger alleviation in Vietnam.

Sheep production, population, distribution and management in Vietnam

The Phan Rang sheep belong to the short-thin tail type and are considered to have an ability to survive in harsh conditions (Mai et al 2003). After years of natural selection and adaptation, the mature weight of the Phan Rang sheep has stabilized at 39 to 45 kg for rams and 34 to 38 kg for ewes. The age at puberty is 5.5 to 6 months. The ewes produce on average 1.55 lambs per year and the length of gestation is 148 to 151 days. The lambing interval is 208 to 262 days and daily live weight gain of lambs in the period from 0 to 12 months of age is 68 g to 73 g/day (Binh et al 2003). According to the data of National Statistics Department of Vietnam, 3.3% of the sheep population can be found in the North and 96.7% in the South of Vietnam (Table 3). The annual growth rate of the number of sheep in 1990 to 2000 was 17.8% per year, 116% between 2000 and 2004, and as high as 268% only between 2004 and 2005 (Vietnam National Statistic Department2000, 2004 and Report of Bureau of Animal Husbandry2006). The sheep breeds and their distribution have also changed rapidly from 2004 until today (Tables 1 and 2).

Table 1. Livestock population and production trends

	Population 1000 heads 2000	Growth rate, %/year 1990 - 2000	Population 1000 heads 2004	Growth rate, %/year 2000 - 2004
Pigs	18,886	15.4	26,144	34.5
Cattle	4,064	13.0	4,908	30.2
Chicken	135,760	16.9	218,153	30.5
Buffalos	2,956	10.3	2,870	-0.8
Goats	525	16.8	1,002	47.2
Sheep	4.5	17.8	21.2	116.5

Vietnam National Statistics Department (2000, 2004)

Table 2. Sheep breeds in Vietnam in 2005.

No.	Sheep breeds	Numbers	% in total
1	Phan Rang	56767	99.9
2	Dorper, Suffolk	60	0.1
Total numbers		*56827*	*100*

Source: Report of Bureau of Animal Husbandry, 2006

According to Binh and Lin (2005), there are three systems of management of small ruminants in Vietnam (intensive, semi-intensive and extensive systems), whereas, the two main systems for management of sheep in Phan Rang are the extensive system and the semi-extensive system (Mai et al 2003). The semi-extensive system can normally be found on large private or state farms, with herd sizes ranging from hundreds to

thousands. In this system, the sheep are allowed to graze during the day time and are supplemented with feeds during the night time. In the extensive system, most sheep are privately owned and are mainly kept by rural smallholders, and the flock size is usually between 10 and 100 head. The sheep are grazed all day and brought back to the house at night time, and are not given any supplementary feed. Some exotic sheep breeds, such as Dorper and White Suffolk, were imported by the government from Australia recently for use in intensive systems in Vietnam. In these systems, the sheep are kept in individual pens and feed are supplied entirely from the outside.

Table 3. The population and distribution of sheep in Vietnam in 2005.

Areas	Numbers of sheep	% of total	Breeds
Khanh Hoa	2100	3.69	Phan Rang
Daklak	105	0.18	Phan Rang
Ben Tre	5000	8.79	Phan Rang
Binh Phuoc	12	0.02	Phan Rang
Binh Thuan	7000	12.32	Phan Rang
Ninh Thuan	41940	73.80	Phan Rang
	60	0.11	Dorper, Suffolk
Dong Nai	110	0.19	Phan Rang
GRRC	500	0.88	Phan Rang
Total number	*56827*	*100*	

Source: Report of Bureau of Animal Husbandry, 2006

The use of some local feed resources for ruminant production

Many experiments on planning, processing, storing and using forages, foliage from multipurpose trees and by-products for small ruminants have been carried out. Several kinds of forage and multi-purpose trees (Table 4), with high biomass and high crude protein have been selected and applied on farm and have proved to be good feed resources for small ruminants, especially in the dry season.

Table 4 Biomass yield (tonnes/ha/year) of some promising forage species in the Bavi region of North Vietnam

Species	Biomass	Dry matter	Crude protein
Flemingia macrophylla	60.7	13.4	2.24
Trichanthera gigantea	62.7	8.1	1.33
Leucaena hybrid KX2	54.8	13.7	2.84
Leucaena leucocephala K636	39.7	9.9	2.1
Leucaena pallida K748	45.2	11.6	2.5
Mulberry (Morus alba)	23.0	3.9	0.67
Bananas (pure stand)	90.7	13.4	-
Trichanthera gigantea in association with banana	82.4	10.6	-
Panicum Maximum cv likoni	75.5	12.8	1.66
Brachiaria ruziziensis	76.9	13.8	1.38
Elephant grass	88.6	15.0	1.75

Nguyen Thi Mui et al (2001)

Advantages and disadvantage of small ruminant production in Vietnam

Advantages of small ruminant production

The rapid expansion of small ruminant production was meeting the policies of the Vietnamese government by creating employment and improving the well-being of poor farmers.
A low investment in breeds, sheds and feeds resulting in high returns to capital invested through short generation cycles and high productive rates.
Efficient use of available pastures in the hilly and mountainous areas which occupy three quarters of the country.
Lamb meat, goat meat and milk are highly appreciated and more expensive than other products, which in turn, can provide higher incomes for producers and better nutrition for humans, particularly malnourished children (comprising 30% of children) and the elderly.

Major constraints of small ruminant production systems

The following constraints to small ruminant production in Vietnam were recognised:
While some appropriate technologies for improving small ruminant production had been developed in the last few years, these technologies were region specific and need to be modified and expanded to meet the needs of all regions of Vietnam, particularly Central and South Vietnam.
Lack of experience and knowledge, availability of credit, technical information and lack of productive breeds severely affect the rate and extent productivity could be improved, especially in hilly and mountainous areas.

Effect of tannins in ruminant digestion

Tannins are the polyphenolic polymers distributed in many plants. A number of forages such as sainfoin (*Onobrychis viciifolia*), Sericea Lespedeza (*Lespedeza cuneata*), *Lotus pedunculatus* (lotus), and*Lotus corniculatus* (Birdsfoot trefoil) contain condensed tannins, which are beneficial for the rumen fermentation when present in moderate quantity (4 to 6% of the total) in the diets. However, high dietary concentrations (6-12% DM) can depress voluntary feed intake, digestive efficiency and animal productivity. Min et al (2003) reported in their review that dietary concentration of condensed tannins, ranging from 2 to 4.5% of total dry matter, improved the efficiency of N use and increased daily weight gain in lambs on temperate fresh forages like *Lotus corniculatus*. One of the reasons for these effects could be an increased metabolizable protein supply, from the protein-binding action of condensed tannins in the rumen when animals are fed a diet with highly degradable protein.

Effect of tannin on methane production

In addition, tannins have been found to decrease methane production, which is beneficial for sparing of energy loss as methane. There was a 16% reduction in methane production in lambs fed on *Lolium pedunculatus*, which is due to the present of condensed tannins (Waghorn et al 2002). Other condensed tannins contained in the forage*Sericea lespedeza* (17.7% CT) decreased methane emission (7.4 vs. 10.6 g/d and 6.9 vs. 16.2 g/kg DMI for *Sericea lespedeza* and crabgrass/tall fescue, respectively) in Angora goats (Puchala et al 2005). Results indicated that condensed tannins action on methanogenesis can be attributed to indirect effects via reduced hydrogen production (and presumably reduced forage digestibility) and via direct inhibitory effects on methanogens.

Effect of urea and nitrate on ruminants

Urea has been the preferred source of non-protein N to supply fermentable nitrogen(ammonia) to ruminants on diets that are deficient in crude protein since McDonald (1948) first demonstrated the key role of ammonia as the N source for microbial protein assimilation. It is now fully accepted that most rumen microbes can use ammonia as a sole N source for cell growth (Allison 1969). Virtanen (1966) demonstrated that urea could be the sole source of nitrogen in a diet fed to dairy cows and therefore microbial cell growth in the rumen could supply all the essential amino acids for cows producing moderate amounts of milk. Just as with urea, nitrate isconverted to ammonia by rumen organisms (Lewis 1951) and is a potent source of ammoniafor bacterial growth.

It has been reported that nitrate and/or reduced N-oxides, such as nitrite, nitric oxide, and nitrous oxide, suppress methane production (Akunna et al 1994; Klüber and Conrad 1998a; Klüber and Conrad 1998b; Clarens et al 1998; El-Mahrouki and Watson-Craik, 2004). The suppression is greatest with nitrous oxide. The apparent non-enzymatic production of nitrogen oxides when nitrate is metabolized in the rumen has implications for the control of methanogenesis (Kaspar and Tiedje 1981).

The predominant pathway of nitrate metabolism in the rumen is uncertain but has always been assumed or even asserted to be dissimilatory nitrate reduction to ammonia; the overall two steps of reduction of nitrate are shown below

$NO_3^- + 2H^+ \rightarrow H_2O + NO_2$ ----------Equation 1

$NO_2^- + 6H^+ \rightarrow H_2O + NH_3$ ----------Equation 2

Organisms capable of nitrite ammonification usually have the ability to reduce nitrate to nitrite in dissimilatory metabolism (Simon 2002), nitrite being a suitable electron acceptor for anaerobic respiration.

Urea is quantitatively and rapidly converted to ammonia in the rumen and the pattern of ammonia accumulation from a urea load in the rumen should be qualitatively similar to that occurring from a nitrate load if dissimilatory nitrate reduction to ammonia is the primary reason for the rapid clearance of nitrate.

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Effect on methane production of supplementing a basal substrate of molasses and cassava leaf meal with mangosteen peel (*Garcinia mangostana*) and urea or nitrate in an *in vitro* incubation

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Abstract

An *in vitro* incubation was used to evaluate effects of Mangosteen peel extract with calcium nitrate or urea on methane production from a substrate of molasses and cassava leaf meal. The design was a 2*2 factorial arrangement in a completely randomized block design with four replications.

Calcium nitrate as replacement for urea, and addition of Mangosteen extract, lowered methane production during the final phase (32 to 48h) of the incubation. Estimates of effects over the entire incubation period, based partially on results from similar experiments in the literature, support the effect of calcium nitrate in lowering methane production. The apparently beneficial effect of Mangosteen peel extract in reducing methane production needs to be substantiated by further research.

Key words: *fermentation, gas production, tannins, saponins*

Introduction

On a worldwide basis, enteric methane from ruminants is estimated to represent 17–30% of total anthropogenic methane (Beauchemin et al 2009). The methane resulting from methanogenesis represents a loss of dietary energy to the animal (from 2 to 12% of the gross energy intake according to Johnson and Johnson (1995) and it is a significant greenhouse gas (Steinfeld et al 2006). According to Hindrichsen et al (2005), 85-90% of methane is produced by enteric fermentation. Murray et al (1976) indicated that 89% of the methane was excreted in the animal's breath and 11% from the anus. These factors have led to a global search for nutritional strategies to mitigate methane emission from ruminants.

The presence of saponins and condensed tannins in forages has been shown to decrease methane production both *in vivo* and *in vitro* (Puchala et al 2005; Holtshausen et al 2009; Szumacher-Strabel and Cieślak 2010). Sainfoin (*Onobrychisviciifolia*), *Lotus pedunculatus* (lotus) and *Lotus corniculatus* (Birdsfoot trefoil) contain condensed tannins which have been shown to be beneficial for the rumen fermentation when they are present in moderate quantity (4 to 6% of the diet DM) in the diets (Patra 2007).

Mangosteen (*Garcinia mangostan*) peel is a fruit by-product which has been used in combination with coconut oil to improve rumen ecology and milk production (Suchitra and Wanapat 2008). According to these authors, mangosteen peel is rich in condensed tannins and crude saponins (16 and 10% in DM, respectively), which they considered to be responsible for the observed reduction in rumen protozoa, and the concomitant increase in rumen bacteria in dairy cattle fed 100 g DM/d of mangosteen peel.

Leng (2007) reported that nitrate can replace carbon dioxide as an electron acceptor with the generation of another reduced product. In this case, ammonia, i.e. nitrate is reduced to nitrite and then to ammonia, resulting in lower methane gas emission. Bozic at al (2009) reported that methane production dropped by 98% in an in vitro test with sodium nitrate. Nolan et al (2010) found that in sheep supplemented with 4% nitrate instead of urea, the reduction of methane production was 23%. Several other studies have now shown that nitrate effectively inhibits methane production (Klüber et al 1998, Guo et al 2009, Guangming et al 2010, Mohanakrishnan at al 2008).

The present investigation was aimed to determine the effects of mangosteen peel on methane production in an *in vitro* incubation with calcium nitrate or urea as the source of non-protein nitrogen and molasses and cassava leaf meal as the basal substrate.

Materials and Methods

Location and duration

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

Treatments and experimental design

Four treatments were compared in a 2*2 factorial arrangement in a completely random block design with four replications. The factors were: with or without mangosteen peel supplement; and urea or calcium nitrate as source of NPN. Individual treatments were:

Mangosteen peel plus calcium nitrate (MP-CaN)
Mangosteen peel plus area (MP-U)
Calcium nitrate (CaN)
Urea (U)

The basal substrate was a mixture of molasses and cassava leaf meal (Table 1).

Table 1. Ingredients in the substrate (g DM)

Items	Urea		CaN	
	MP	No MP	MP	No MP
Molasses	8.68	8.76	8.46	8.54
Cassava leaf meal	3.00	3.00	3.00	3.00
Urea	0.24	0.24		
$Ca(NO_3)_2.4H_2O$			0.46	0.46
Mangosteen peel	0.08		0.08	

The *in vitro* system

The *in vitro* system using recycled water bottles (Photo 1) has been described in detail by Sangkhom et al (2011).

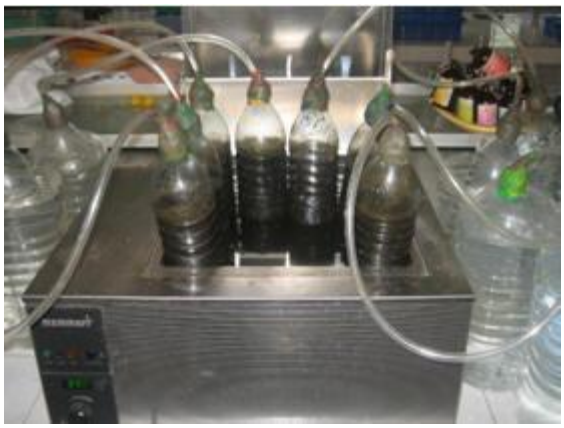

Photo 1. The *in vitro* fermentation system

Photo 2. Measurement of percentage of methane with the Crowcom meter

Preparation of substrate and rumen fluid

The forage component of the diet (cassava leaf and mangosteen peel) was cut into small pieces, about 1 cm of length, dried at 65°C during 24h and then milled in a coffee grinder, prior to mixing with molasses and the source of NPN (urea or calcium nitrate). Representative samples (Table 1) of the mixtures (12g DM) were put into the incubation bottle to which were added 0.96 liters of buffer solution (Table 2) and 0.24 liters of rumen fluid, prior to filling each bottle with carbon dioxide. The rumen fluid was taken at 10-11pm in 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 8-9am the following morning when the contents were filtered through one layer of cloth before being added to the incubation bottle. The bottles were then incubated at 39°C for 48h.

Table 2. Ingredients of the buffer solution (adapted from Tilly and Terry 1963)

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

Photo 3. Mangossteen fruit

Photo 4. Cassava for foliage production

Measurements

Gas volume and the content of methane was recorded during periods of incubation from 0-8h, 9-21h, 22-31h and 32-48h. The methane content of the gas was measured by pasing the gas sample through an infra-red meter (Crowcon Ltd, UK; Photo 2). At the end of the incubation the total gas volume was calculated and the residual DM in the incubation bottle measured by filtering through cloth and oven-drying at 100°C for 24h.

Statistical analysis

Data were analyzed by the General Linear Model in the ANOVA program of the MINITAB software (Version 13.2; Minitab 2000). Sources of variation in the model were: Mangosteen peel, NPN source, interaction Mangosteen*NPN source and error.

Results and discussion

Effects of dietary mangosteen peel and NPN sources on gas production and concentration of methane

Gas production was not affected by Mangosteen peel but was reduced in the incubations 0-8h, 9-21h and overall 90-48h, when calcium nitrate rather than urea was the NPN source (Table 3). Methane concentration in the gas in the incubation 0-8h was reduced by Mangosteen peel (Figure 2) and when calcium nitrate rather than urea was the NPN source (Figure 1). The effect of the Mangosteen peel was seen at all incubation times as was that of calcium nitrate, with the exception of the 22-31h period (Table 3). The proportion of the substrate fermented was reduced when calcium nitrate rather than urea was the NPN source, but was not affected by supplementation with Mangosteen peel. Overall production of methane per unit of substrate fermented was reduced by supplementation with mangosteen peel and by calcium nitrate as opposed to urea.

Table 3. Mean values for gas production, percentage of methane in the gas, substrate fermented and methane production per substrate fermented according to effect of additive (mangosteen peel MP) and source of NPN

	Additive			NPN source			
	MP	**No MP**	**P**	**CaN**	**Urea**	**P**	**SEM**
Gas production, ml							
0-8h	508	560	0.135	424	644	0.001	23
9-21h	253	227	0.32	180	299	0.001	17.6
22-31h	323	351	0.2	326	348	0.335	14.8
32-48h	289	258	0.35	242	305	0.69	22
Total	1371	1396	0.65	1173	1595	0.001	38.2
% Methane							
0-8h	9.63	14.21	0.001	9.71	14.1	0.001	0.55
9-21h	22.9	28.2	0.00	21.5	23.0	0.065	0.53
22-31h	23.5	33.6	0.001	24.7	26.4	0.118	0.7
31-48h	20.0	24.5	0.001	25.3	31.8	0.001	0.95
0-48h	17.6	23.1	0.001	18.8	21.8	0.001	0.40
DM fermented after 48h, %	50.8	53.8	0.23	49.4	55.2	0.035	1.7
Methane, ml/g DM fermented	39.5	49.7	0.006	36.7	52.5	0.001	2.05

The beneficial effects in reduction of methane production when calcium nitrate replaced urea as the NPN source in an in vitro incubation are similar to those reported by Phommasack Outhen et al (2011), Sangkhom et al (2011) and Le Thuy Binh Phuong et al (2011). There are no reports of the effect of Mangosteen peel on methane production in an *in vitro* incubation. Reduced production of methane was reported by Khan and Chaudhry (2009) for a range of spices added to an in vitro incubation, especially for Coriander (*Coriandrum sativum*) and in the latter case was attributed to the high levels of tannins reducing methanogenisis and/or the uptake of hydrogen for bio-hydrogenation of the unsaturateed fatty acids. Presumably, the high levels of saponins and tannins reported in Mangosteen peel (Suchitra and Wanapat 2008) were the determinant factors in bringing about the reduction in methane in the present study.

In the report of Khan and Chaudhry (2009), Coriander was found to lower methane production from 14ml/g original substrate to 8ml/g substrate after 24h incubation – a drop of 40%. In our study the lowering of methane with Mangosteen peel after 21h incubation was from 11.4 to 5.5 ml/g substrate (a drop of 51%) while the reduction with calcium nitrate versus urea was from 15.2 to 7.4 ml/g of substrate (also a drop of 51%).

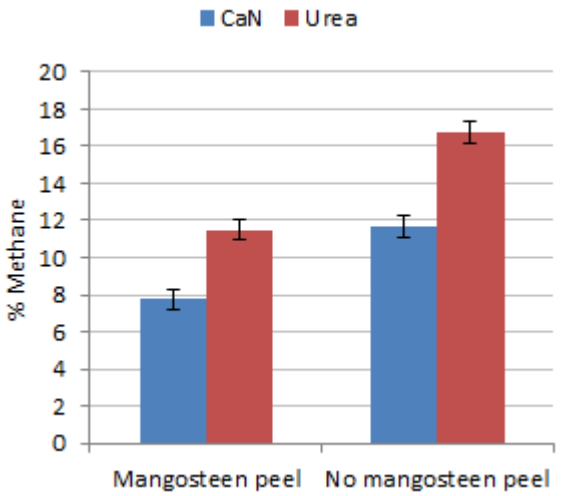

Figure 1. Effect of calcium nitrate or urea as source of fermentable N on percent methane in the gas after 8h of fermentation in an *in vitro* system with substrate of molasses and cassava leaf meal with or without mangosteen peel extract

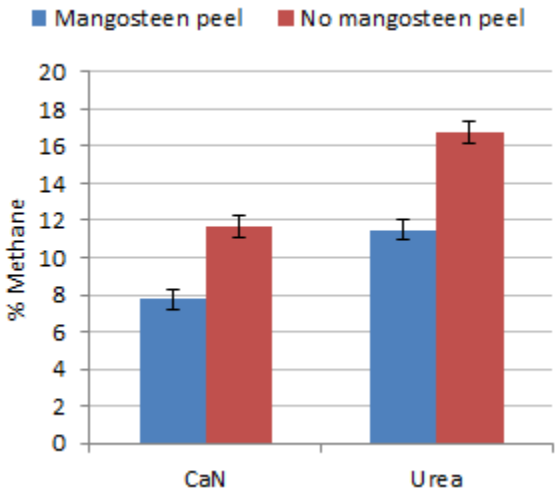

Figure 2. Effect of mangosteen peel extract on percent methane in the gas after 8h of fermentation in an *in vitro* system with substrate of molasses and cassava leaf meal, with calcium nitrate or urea as source of fermentable N

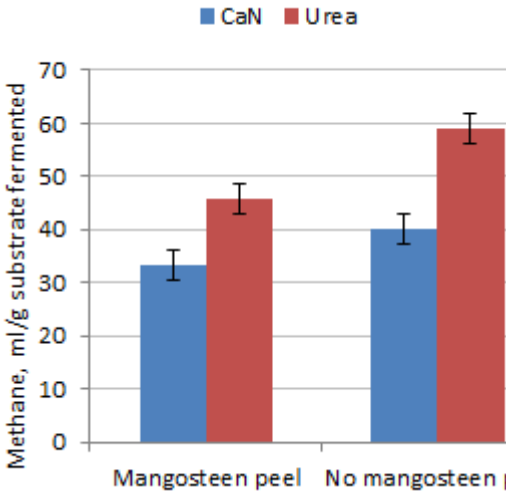

Figure 3. Effect of calcium nitrate or urea as source of fermentable N on methane production per unit substrate fermented in an *in vitro* system with substrate of molasses and cassava leaf meal with or without mangosteen peel extract (48h incubation)

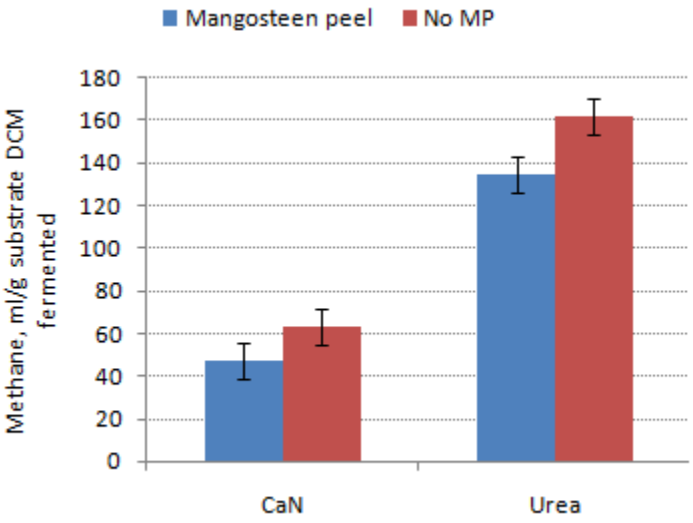

Figure 4. Effect of mangosteen peel extract on methane production per unit substrate fermented in an *in vitro* system with substrate of molasses and cassava leaf meal and calcium nitrate or urea as source of fermentable N (48h incubation)

The concentration of methane in the gas increased with curvilinear trends with time of incubation, the pattern being similar for the effects of the NPN source and for the Mangosteen peel additive (Figures 5 and 6). As discussed by Sangkhom et al (2011), these changes almost certainly reflected changes in the nature of the fermentation as carbohydrate was fermented first to VFA, methane and carbon dioxide followed by secondary fermentation of the VFA to methane and carbon dioxide.

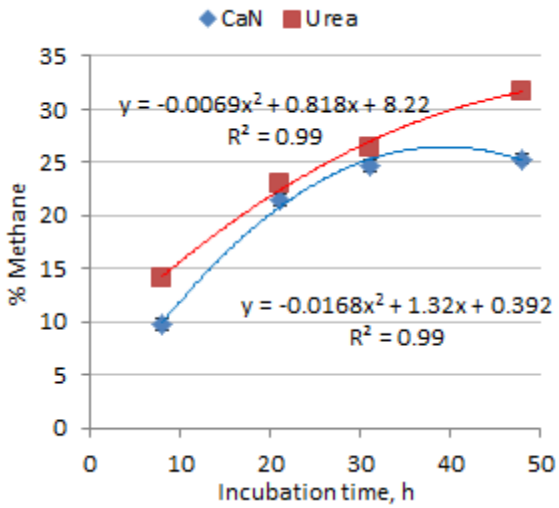

Figure 5. Effect of incubation time on methane content of the gas with nitrate or urea as the NPN source

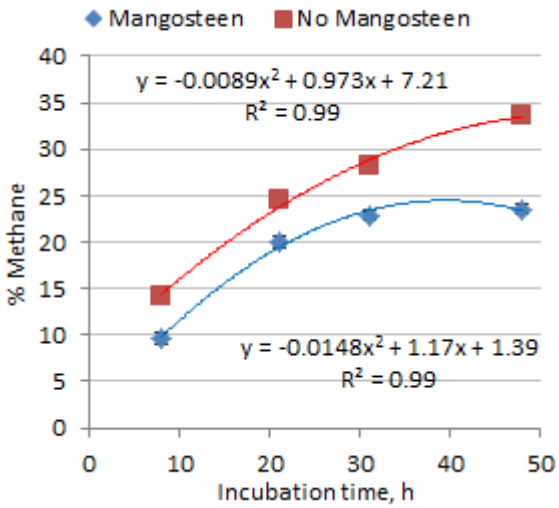

Figure 6. Effect of incubation time on methane content of the gas with and without addition of Mangosteen peel

Conclusions

Calcium nitrate as replacement for urea, and addition of Mangosteen extract, lowered methane production an *in vitro* incubation of molasses and cassava leaf meal.

The apparently beneficial effects of Mangosteen peel in reducing methane production needs to be substantiated by further research.

Acknowledgements

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Effect of potassium nitrate or urea as NPN source and levels of Mangosteen peel on *in vitro*gas and methane production using molasses, *Operculina turpethum*and *Brachiaria mutica* as substrate

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Abstract

The objective of this study was to evaluate the effect of the levelof Mangosteen peel and potassium nitrate or urea as non-protein nitrogen source on methane production in an *in vitro* incubation. The design wasa 3*4 factorial with 3 replicates. The factors were source of non protein nitrogen: urea (1.83% of substrate, DM basis) and potassium nitrate (4 or 6% of substrate, DM basis); and levels of Mangosteen peel (0, 0.5, 1 and 1.5% of substrate DM basis). The quantity of substrate was 2.5g to which were added 200ml of buffer solution and 50ml of buffalo rumen fluid taken immediately after the animal was killed in the slaughter-house. The incubation was for 48 h with measurements of gas and methane production at 6, 12, 24, 36 and 48 h. The proportion of substrate solubilized at 48h was determined by filtration, followed by measurement of ammonia-nitrogen concentration in the filtrate.

After 48h incubation, gas and methane production, per cent substrate DM digested and methane produced per unit DM digested, were lower when potassium nitrate was the NPN source compared with urea. The 6% level of potassium nitrate was more effective in reducing methane production than the 4% level. Gas and methane production increased with time of incubation. Similar reductions in the above parameters were observed with increasing level of Mangosteen peel in the substrate. The ammonia concentration in the filtrate after 48h of incubation was lower when potassium nitrate was the NPN source compared with urea.

Keywords: *Climate change, greenhouse gases, incubation, rumen.*

Introduction

Due to the World challenging problems of energy and food crisis, and negative effects of global climate changes which livestock production is facing, particularly the animal nutrition must consider not only nutrient requirements and production but also friendly environment, sustainable productivity and human and animal welfare. Preston (2009) concluded that instead of conflict in the use of biomass for food and fuel, there can be synergism, and that, instead of contributing to climate change, farming systems can have a negative carbon footprint. Hindrichsen et al (2005) indicated that 85-90% of methane is produced by enteric fermentation and Murray *et al* (1976) also concluded that 89% of the methane is excreted in the animal's breath and 11% from the anus. There is a great incentive to reduce methane emissions from livestock.

Mangosteen (*Garcinia mangostana*) is a tropical plant which produces an edible fruit; it is very familiar with people in Southeast Asia. The fruit when ripe has a thick outer skin, of dark purple color. The Mangosteen peel contains both condensed tannins and crude

saponins, which exert a specific effect against rumen protozoa, while the rest of the rumen biomass remains unaltered (Ngamsaeng and Wanapat 2005). Ngamsaeng and Wanapat (2005) suggested that supplementation of mangosteen peel (100g DM/d) in cattle can increase the population of rumen bacteria and decrease the protozoal population, and maintain the fungal zoospore population.

Tannins have been found to inhibit methane in the rumen fermentation, which is beneficial for sparing of energy loss by this route. Many types of forage are known to contain condensed tannins and have been shown to decrease methane production both *in vivo* and *invitro* such as sainfoin (*Onobrychisviciifolia*), *Lotus pedunculatus* (lotus) and *Lotus corniculatus* (Birdsfoot trefoil) (Patra 2007). Condensed tannins are beneficial for the rumen fermentation when they are present in moderate quantity (4 to 6% of the total DM) in the diets (Patra 2007). Woodward et al (2001) investigated the feeding of sulla (*Hedysariumcoronarium*) on methane emission and milk yield in Friesian and Jersey dairy cows. Cows grazing on sulla had higher daily dry matter intake (13.1 vs. 10.7 kg DM) and daily milk solid production (1.07 vs. 0.81 kg) than when grazing on perennial ryegrass pasture. Total daily methane emission was similar (254 vs 260 g).

Thepresentinvestigation aimed to determine the effects of potassium nitrate or urea as the source of non-protein nitrogenonmethane production in an *in vitro* incubation in which there were various levels of mangosteen peel.

Hypothesis

The hypothesis to be tested was:

Potassium nitrate and mangosteen peel will reduce methane production in an *in vitro* incubation using molasses and *Operculina turpethum* as the basal substrate.

Materials and methods

Location and duration

The *in vitro* fermentation was conducted in the laboratory at the Department of Animal Sciences, Faculty of Agriculture and Applied Biology, Cantho University, Cantho city, Vietnam.

The experiment was donefrom May to July, 2011.

Experimental design

Thiswas a 3*4 factorial design with 3 replicates. The factors were
Sources of non protein nitrogen: 1.83% urea, 4% and 6% potassium nitrate
Mangostgeen peel: 0%, 0.5%, 1.0% and 1.5% (DM) of the substrate.
The basal substrate was a mixture of 35% (DM) para grass, 35% (DM) *Operculina turpethum* and22.5% to 28.2% (DM) molasses.

The *in vitro* system

The *in vitro* system was based onthe same procedure (Photos 1 and 2) that has been described in an earlier paper (Thanh et al 2011).

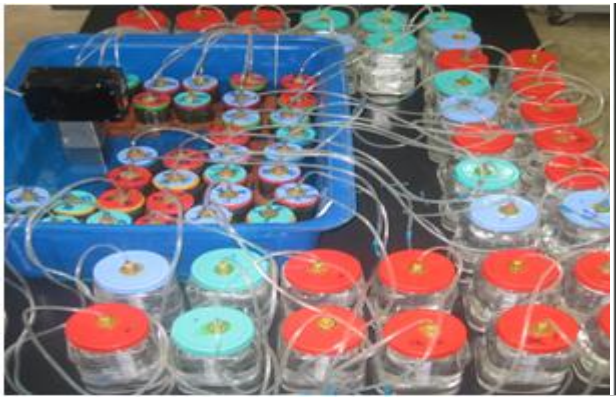

Photo 1. The *in vitro* system

Photo 2. Measurement of percentage of methane with the Crowcon meter

Preparation of substrate and rumen fluid

The *Operculina turpethum*, para grass and mangosteen peel were cut into small pieces, about 1 cm of length and then dried at 65°C during 24h. Representative samples of the mixtures (2.5g DM) were put into the incubation bottle to which were added 0.2 liters of buffer solution (Table 1) and 0.05 liters of rumen fluid, prior to filling each bottle with carbon dioxide. The rumen fluid was taken at 9-10am in the slaughter-house from a buffalo immediately after the animal was killed. Representative samples of the rumen were filtered through two layers of cloth (for keeping feed residues in the rumen fluid) before being added to the incubation bottle.The flasks were then incubated at 38°C for 48h.

Table 1. Ingredients of the buffer solution

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

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

Measurements

The incubation was for 48 h with measurements of gas and methane production being recorded at 6 h, 12 h, 24 h, 36 h and 48 h of incubation. The methane percentage of the gas was measured by passing the gas sample through an infra-red meter (Crowcon Ltd, UK; Photo 2). Unfermented solids at 48 h were determined by filtering through two layers of cloth and drying at 100°C for 24h. Ammonia-nitrogen (NH_3-N) concentration was measured in the filtrate.

Chemical analyses

The ingredients in the substrate were analysed for DM, OM, CP and ash according to the standard methods of AOAC (1990).

Statistical analysis

Data were analyzed by the General Linear Model in the ANOVA program of the MINITAB software (Version 13.2; Minitab 2000). Sources of variation in the model were: Levels of Mangosteen peel, NPN sources, interaction levels of Mangosteen peel*NPN source and error.

Results

Chemical composition

Both Mangosteen peel and *Operculina turpethum*had higher crude protein than the para grass (Table 2).

Table 2. Chemical composition of substrate ingredients of experiment 1

Ingredients	DM	OM	CP	Ash
Para grass	18.4	87.7	8.58	12.3
Operculina turpethum	9.75	86.0	15.5	14.0
Molasses	78.0	93.1	3.39	6.88
Mangosteen Peel	82.3	95.3	18.1	4.67
Urea			282	
KNO_3			86.9	

In vitro gas and methane production

After 48h incubation, gas and methane production, per cent substrate DM digestedand methane produced per unit DM digested, were lower when potassium nitrate was the NPN source compared with urea (Table 3). The 6% level of potassium nitrate was more effective in reducing methane production than the 4% level. Gas and methane production increased with time of incubation (Figures 1 and 2). Similar reductions in the above parameters were observed with increasing level of Mangosteen peel in the substrate (Figures 4 to 6).

Table 3. *In vitro* gas, methane production and digestible substrate affected by source of NPN and mangosteen peel at 48 hour

	NPN			MP				P		
	UREA	KN4	KN6	MP0	MP0.5	MP1	MP1.5	NPN	MP	NPN*MP
Gas production, ml	307^a	262^b	208^c	308^a	264^b	233^c	230^c	<0.001	<0.001	0.087
CH_4, %	9.01^a	3.63^b	2.54^c	5.78^a	5.13^b	5.18^b	4.14^c	<0.001	<0.001	<0.001
CH_4, ml	28.0^a	9.51^b	5.42^c	19.3^a	14.5^b	13.1^b	10.3^c	<0.001	<0.001	<0.001
DMD, g	1.82^a	1.73^b	1.69^b	1.76	1.74	1.74	1.73	<0.001	0.828	0.867
DMD, %	71.7^a	69.2^b	67.2^c	70.1	69.8	69.0	68.7	<0.001	0.33	0.993
CH_4/DMD, ml/g	15.4^a	5.49^b	3.17^c	10.8^a	8.09^b	7.29^b	5.92^c	<0.001	<0.001	<0.001

abc Means within main effects without common superscript differ at P<0.05

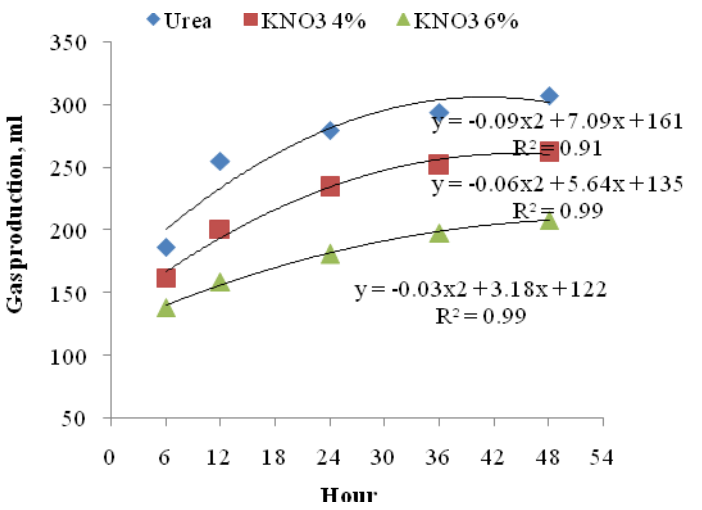

Figure 1. Potassium nitrate or urea as source of non protein nitrogen on gas production at 48 h

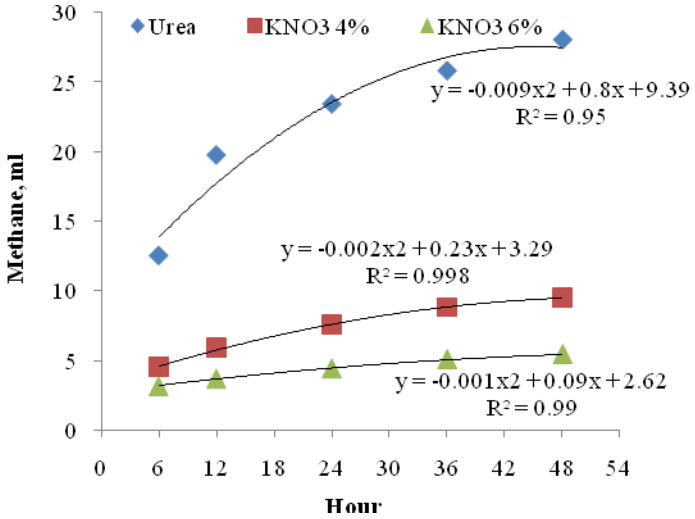

Figure 2. Potassium nitrate or urea as source of non protein nitrogen on methane production at 48 h

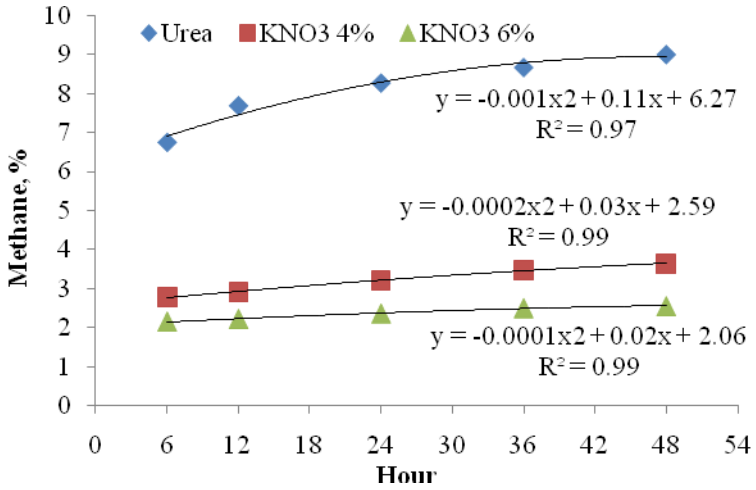

Figure 3. The percentage of methane in the gas with potassium nitrate or ureasource of non-protein nitrogen

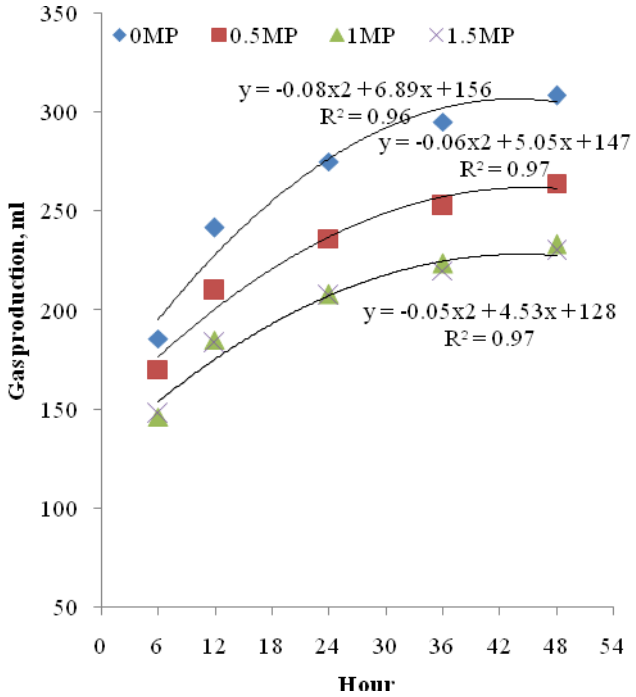

Figure 4. Effect of increasing concentrations of Mangosteen peel on gas production at 48 h fermentation

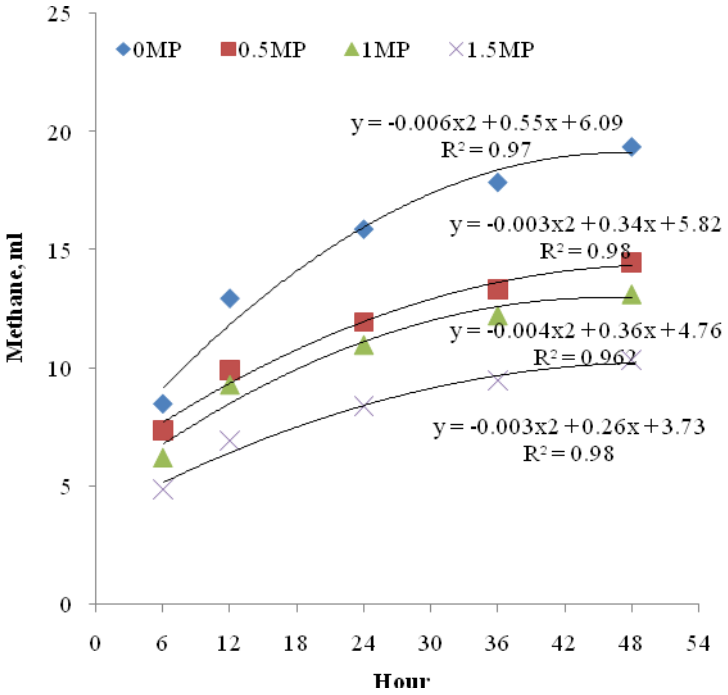

Figure 5. Effect of increasing concentrations of Mangosteen peel on methane production at 48 h fermentation

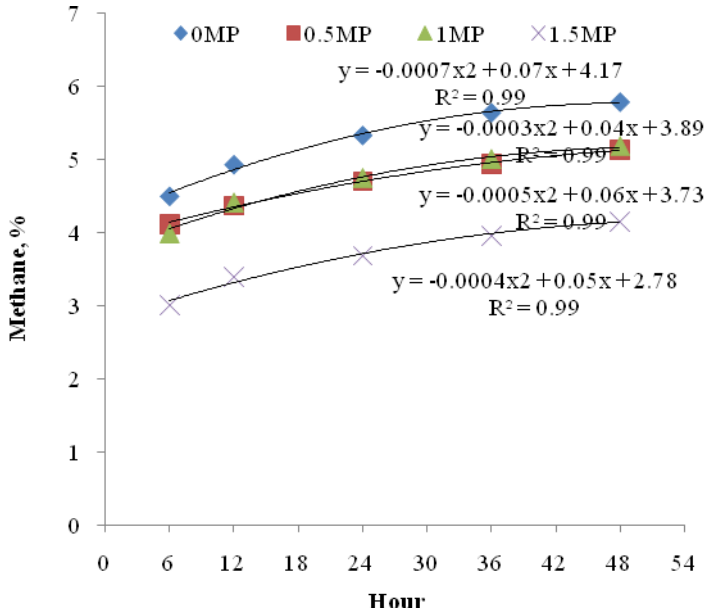

Figure 6. Effect of incubation time and increasing concentrations of mangosteen peel on methane per cent in the gas

There were close negative relationships between the level of Mangosteen peel in the substrate and methane production (Figures 7-8) and the per cent of substrate solubilized (Figure 9).

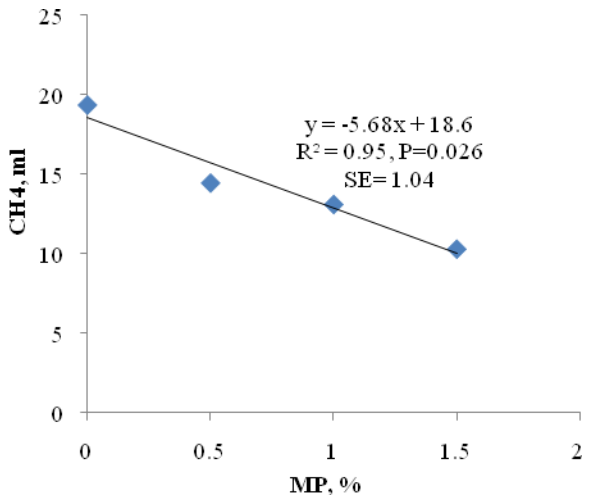

Figure 7. Relationship between level of mangosteen peel and methane production

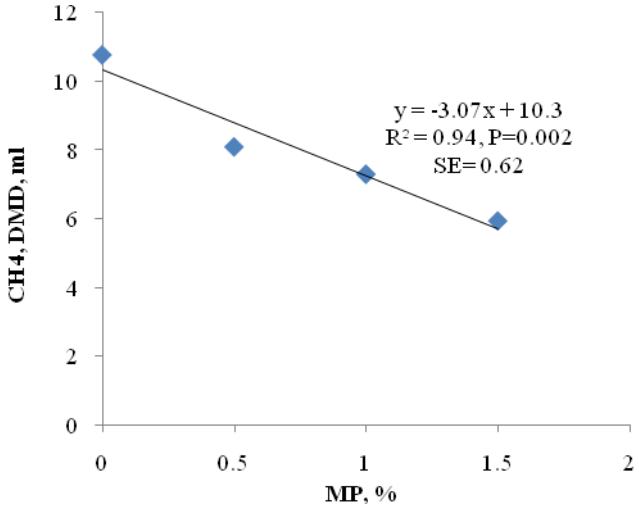

Figure 8. Relationship between level of mangosteen peel and methane productionper unit substrate DM fermented

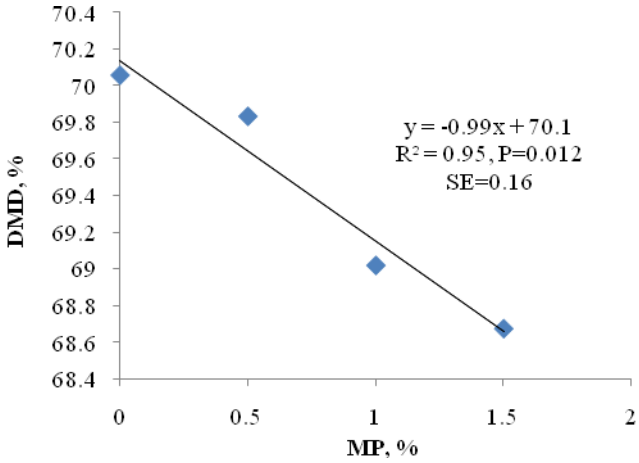

Figure 9. Effect of mangosteen peel on digested substrate

The ammonia concentration in the filtrate after 48h of incubation was lower when potassium nitrate was the NPN source compared withurea (Table 4). There were no differences in pH, the values of which were normal for fermentation by rumen micro-organisms

Table 4. Mean values for pH and ammonia in the filtrate after 48h incubation

	NPN			MP				SEM		P		
	UREA	KN4	KN6	MP0	MP0.5	MP1	MP1.5	NPN	MP	NPN	MP	NPN*MP
pH	6.77	6.74	6.79	6.78	6.74	6.77	6.78	0.015	0.018	0.088	0.506	0.122
N-NH_3 mg/100ml	24.6a	16.9b	15.6b	19.9ab	21.8a	17.4ab	17.1b	0.9899	1.1431	<0.001	0.025	0.004

abc Means within main effects without common superscript differ at P<0.05

Discussion

The beneficial effects of mangosteen peel in reducing methane productionin an *in vitro* incubation were similar to those reported by Thanh et al (2011). In the earlier study, the

level of Mangosteen peel was 0.67%. The results of the present experiment indicate that higher levels of Mangosteen peel, up to 1.5%, can be used with greater reduction in methane and without apparent negative effects on the fermentation. Similar positive effects on methane reduction were reported by Khan and Chaudhry (2009) for a range of spices added to an *in vitro* incubation, especially for coriander (*Coriandrum sativum*). These authors attributed the reduction in methane to the high levels of tannins reducing methanogenisis and/or the uptake of hydrogen for bio-hydrogenation of the unsaturated fatty acids.

The positive effect of potassium nitrate in reducing methane production in an vitro incubation Leng (2007) is in line with the results of many similar studies with similar or different substrates (Outhen et al 2011; Sangkhom Inthapanya et al 2011; Phuong et al 2011) when nitrate salts replaced urea as the source of NPN.

Conclusions

Methane production in an *in vitro* incubation was reduced by potassium nitrate compared with urea and by increasing levels of Mangosteen peel.

The present of potassium nitrate and mangosteen peel resulted in reduction of digested substrate in an *in vitro* system.

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Effect of potassium nitrate or urea as NPN sources associated with Mangosteen peel (*Garcinia mangostana*) on methane production, rumen parameters and growth performance of Phan Rang sheep in the Mekong Delta of Vietnam

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Abstract

Twelve female Phan Rang sheep with an initial weight of 21.3 ± 0.2 kg at 4 months of age were allocated in a 2 x 2 factorial design with 3 replicates. The first factor was non protein nitrogen source (urea or potassium nitrate); the second factor was Mangosteen peel meal at 0 or 1.5% of the diet DM.

Feeding potassium nitrate rather than urea decreased the ratio of methane to carbon dioxide in the eructed air from the sheep. There was a tendency for methane production to be reduced by supplementation with Mangosteen peel. There was no effect of the NPN source, nor of the supplementation with Mangosteen peel, on apparent digestibility, N retention, and growth performance.

***Key words**: Ammonia, digestibility, climate change, feed conversion, greenhouse gases, live weight gain, VFA*

Introduction

Sheep husbandry is practiced throughout the majority of the inhabited world, and has been fundamental to many civilizations. However, as for other ruminants sheep produce methane which has 21 times the impact of carbon dioxide in forcing global warming (The CattleSite News Desk, 2000). Therefore developing technologies of reducing greenhouse gas emissions from the rumen is very important for sheep production. Particularly the Mekong delta is one of the regions in the world, which is seriously vulnerable with the global climate changes and predicted sea level rise.

In a recent review, Leng (2008) 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. As reported previously by Trinh Phuc Hao et al (2009) and Le Thi Ngoc Huyen et al (2010) nitrate could be fed safely to goats and cattle as the source of NPN for the rumen fermentation. Several studies have now shown that nitrate effectively inhibits methane production (Nolan et al 2010, Jeong et al 2005, Bozic et al 2009, Kluber and Conrad 1998, Guo et al 2009, Guangming et al 2010, Mohanakrishnan et al 2008).

Previous studies with Mangosteen peel were aimed at modifying the rumen fermentation (Suchitra Kanpukdee and Wanapat 2008). Supplementation with Mangosteen peel decreased the acetic: propionic acid ratio and it was predicted that this would have resulted in a reduction in methane production. Currently, in Vietnam Mangosteen is mainly distributed in the Mekong Delta with a total area of 4,900ha with a total yield of fruit of about 4,500 tonnes (about 1 tonne/ha)

(http://www.rauhoaquavietnam.vn). The peel of the Mangosteen peel is mostly thrown away in the canals causing pollution of the environment. Therefore its utilization for purposes such as animal production could be beneficial economically and environmentally.

There have been no studies on the effect of Mangosteen peel on rumen methane production in sheep. Phan Rang sheep have been mainly raised in Ninh Thuan province of Vietnam for meat production by the Cham ethnic group. In recent years, sheep production in Mekong Delta has been also developed because the ecological conditions and feed resources in the region are suitable.Thepresentinvestigation was therefore aimed to determine the effects on methane production, rumen parametersand growth in Phan Rang sheep of supplementing the basal diet of molasses and para grass with Mangosteen peel in combination with potassium nitrate or urea as source of NPN.

Materials and methods

Location and duration

The experiment was carried out at the farm of Cantho University, Cantho City, Vietnam, from September to December, 2011.

Experimental design

Twelve female Phan Rang sheep with an initial weight of 21.3 ± 0.2 kg at 4 months of age were used for this experiment. They were allocated in a 2 x 2 factorial design with 3

replicates. The first factor was non protein nitrogen source (urea or potassium nitrate); the second factor was Mangosteen peel meal (0 or 1.5% of diet DM).

Animals and housing

The sheep were kept in individual pens. Vaccination was done againstepidemicdiseasesand the sheep were drenched againstinternalparasitesbeforethecommencementoftheexperiment.

Feeding and management

The diets (Table 1) were introduced gradually during a 14 day period of adaptation, followed by 90 days for the feeding trial. The sheep were fed two times a day (8:00 and 14:00). Clean fresh water was available at all times. The NPN salts were dissolved in the molasses which was fed in two equal portions in the morning (08:00) and afternoon (14:00). The Mangosteen peel was mixed with the soybean meal and offered in two equal portions after feeding the molasses. The *Operculina turpethum*was fed in the morning and the Para grass in the afternoon following the other feeds. Both forages were chopped to a length of 2-3 cm and fed in the fresh state.

Table 1. Composition of the diets (% DM basis)

	Urea (MP0)	**K-Nitrate (MP0)**	**Urea (MP1.5)**	**K-nitrate (MP1.50**
Para grass	44.2	35.8	41.0	39.0
Operculina turpethum	12.5	12.5	12.5	12.5
Molasses	15.9	15.8	15.3	16.3
Soybean meal	24.8	31.2	28.2	27.8
Mangosteen peel	0.00	0.00	1.50	1.50
KNO_3	0.00	4.00	0.00	4.00
Urea	1.8	0.0	1.8	0.00
Total	100	100	100	100
Proximate analysis, %				
OM	87.5	85.8	86.6	86.7
CP	22.3	22.5	22.4	22.4
NDF	39.3	34.9	37.3	36.8
ADF	23.1	21.6	22.3	22.4
Ash	9.56	9.12	9.45	9.23

Measurements

Methane, CO_2, pH, NH_3-N and Volatile fattyacid (VFA)

During the feeding trial period, rumen samples weretaken onday 45 and 85 at 1hourbeforefeedingand at 3hoursafterfeedingforanalyzingpH, NH_3-N, andVFA. The methane: carbon dioxide ratio was determined at 85 days of the experiment using the Gasmet equipment (Photo 9; GASMET 4030 Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland). Rumen volatile fatty acids (VFA) were determined by the procedure of Barnett and Reid (1957), while rumen pH was measured using a glass

electrode pH meter. Rumen ammonia concentration was determined by distillation and titration with 0.1N sulphuric acid (http://mekarn.org/labman/Amoniac.htm).

Sampling procedure for feeds and feces

Feeds offered and refusals were measured daily to calculate feed intake. Feces and urine were collected separately during 5 days from 45 to 50 days and from 85 to 89 days of the experiment. H_2SO_4 was added to the urine container to avoid gaseous ammonia losses during the sampling period.

Feed samples and feces were analyzed for DM, Ash and N, and urine analyzed for N, according to the standard methods of AOAC (1990).

Digestibility and nitrogen balance

Apparent digestibility coefficients for DM, OM and NDF and ADF were determined by the methods described by McDonald et al (2002).

Live weight change

The sheep were weighed in the morning prior to feeding, at the beginning and end of each week during the experimental period.

Statistical analysis

The data wereanalyzed using the General Linear Model option in the ANOVA program of the Minitab (2000) software. The sources of variation were NPN source, Mangosteen peel, interaction NPN*Mangosteen peel and error.

Results and discussion

Chemical composition

The chemical composition of the feed ingredients is shown Table 2.

Table 2. Chemical composition of feed ingredients

Ingredients	DM	OM	CP	EE	NDF	ADF	Ash
	(%)	(% in DM)					
Para grass	18.7	87.7	10.2	4.29	66	31.7	12.3
Operculina turpethum	9.7	86	13.6	6.5	38.8	30.7	14
Molasses	73.6	93.1	2.72				6.88
Soybean meal	83.2	90.6	42	2.43	28.7	19.2	9.4
Mangosteen Peel	82.3	95.3	18	4.09	61.9	60.3	4.67
Urea	100		282				
KNO_3	100		86.9				

DM: Dry Matter, OM: Organic matter, CP: Crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber

Feed intakes

DM intake was reduced when potassium nitrate was the NPN source compared with urea, but was not affected by the feeding of Mangosteen peel (Table 3).

Table 3. Feed intake of sheep (g DM/head/day) in the experiment

	NPN		Mangosteen peel		P		
	Urea	KN	NonMP	MP	NPN	MP	MP*NPN
Molasses	57.7	47.9	49.7	55.9	0.001	0.001	0.350
Operculina turpethum	60.4	62.2	64.0	58.6	0.698	0.265	0.531
Para grass	273	214	233	253	0.002	0.163	0.785
Soybean	108	124	119	112	0.001	0.025	0.005
Total DM	517	478	486	509	0.045	0.200	0.253

Apparent digestibility

There were no differences in coefficients of apparent digestibility of DM, OM, CP, NDF and ADF due to source of NPN or supplementation with Mangosteen peel after 45 and 85 days of the feeding trial (Tables 4 and 5). However, the apparent digestibility of CP was higher at the end of the feeding trial (85 days) than at the midway point (45 days) (Table 6).

Table 4. Coefficients of apparent digestibility in sheep fed forage diets supplemented with nitrate or urea as NPN source and with or without Mangosteen peel (MP) after 45 days of the feeding trial

	NPN			Mangosteen peel			
	Urea	KNO_3	P	No-MP	MP	P	SEM
DM	60.1	58.6	0.74	58.4	60.3	0.68	3.17
OM	61.2	59.2	0.65	59.2	61.2	0.60	3.10
CP	50.2	46.1	0.12	47.5	48.8	0.77	1.59
NDF	55.6	50.8	0.45	52.3	54.1	0.97	4.29
ADF	45.9	37.7	0.34	41.6	42.0	0.94	5.76

Table 5. Coefficients of apparent digestibility in sheep fed forage diets supplemented with nitrate or urea as NPN source and with or without Mangosteen peel (MP) after 85 days of the feeding trial

	NPN			Mangosteen peel			
	Urea	KNO_3	P	No-MP	MP	P	SEM
DM	62.1	59.2	0.49	61.5	59.7	0.66	2.80
OM	63.1	60.2	0.46	62.3	61.0	0.74	2.73
CP	54.6	51.3	0.16	53.6	52.3	0.56	1.50
NDF	58.5	59.2	0.89	59.0	58.7	0.95	3.18
ADF	47.4	43.9	0.63	46.7	44.7	0.79	4.97

Table 6. Coefficients of apparent digestibility in sheep fed forage diets supplemented with nitrate or urea as NPN source and with or without Mangosteen peel (MP) after 45 and 85 days of the feeding trial

	45 days	85 days	SEM	P
DM	59.3	60.6	1.9	0.63
OM	60.2	61.7	1.9	0.58
CP	48.1	52.9	1.3	0.02
NDF	53.2	58.8	2.4	0.10
ADF	41.8	45.7	3.4	0.43

In contrast with the findings in the present study, Diaz et al (1993) and Klita et al (1996) demonstrated that high levels of saponins and/or tannins in diets resulted in decreased apparent digestibility, especially of crude protein. The reason for these differences may lie in the concentration and the source of the saponins/tannins used by these workers. In the study of Ngamsaeng and Wanapat (2005) it was also concluded that tannins decreased protein degradability by complexing with feed protein, which may lead to inhibition of protein degradation in the rumen.

Rumen parameters

In general, the parameters of rumen fermentation were within the normal range for ruminants fed tropical forage diets (Nguyen van Thu 2010).Supplementation with Mangosteen peel and with nitrate or urea as source of NPN had no effect on rumen pH, ammonia or total VFA at the midpoint of the experiment (Table 7). However, after 85 days rumen ammonia was lower with nitrate than urea supplementation (Table 8).

Table 7. Mean values for rumen pH, ammonia and volatile fatty acids at the middle of the experiment (45 days feeding trial)

	NPN		**Mangosteen peel**		**SEM**	**P**		
	Urea	KN	NonMP	MP		NPN	MP	NPN*MP
pH at 0h	6.52	6.47	6.44	6.55	0.042	0.541	0.821	0.916
pH at 3h	6.32	6.33	6.28	6.36	0.023	0.618	0.038	0.215
N-NH3 at 0h, mg/100 ml	31.7	29.6	30.7	30.6	0.961	0.539	0.127	0.548
N-NH3 at 3h, mg/100 ml	42.3	41.6	42.1	41.8	0.756	0.149	0.924	0.776
VFA at 0h, mmol/L	86.4	84.9	83.6	87.7	1.690	0.395	0.101	0.13
VFA at 3h, mmol/L	112	109	110	111	2.354	0.446	0.923	0.846

Table 8. Rumen pH, ammonia and volatile fatty acids at the end of the experiment (85 days feeding trial)

	NPN		**Mangosteen peel**		**SEM**	**P**		
	Urea	KN	NonMP	MP		NPN	MP	NPN*MP
pH at 0h	6.58	6.60	6.61	6.57	0.022	0.647	0.191	0.45
pH at 3h	6.27	6.31	6.30	6.28	0.036	0.437	0.682	0.594
N-NH3 at 0h, mg/100 ml	32.3	28.9	30.4	30.8	1.106	0.066	0.805	0.595
N-NH3 at 3h, mg/100 ml	43.4	41.7	42.5	42.6	0.497	0.049	0.890	0.927
VFA at 0h, mmol/L	89.4	86.8	86.9	89.3	1.298	0.200	0.233	0.874
VFA at 3h, mmol/L	119	115	117	118	2.424	0.242	0.707	0.576

Nitrogen balance and daily weight gain

The dietary treatments had no effect on N retention, live weight gain or DM feed conversion (Table 9).

Table 9. Nitrogen intake, live weight and feed conversion of sheep in experiment 2

	NPN		Mangosteen peel		SEM	P		
	Urea	KN	No MP	MP		NPN	MP	NPN*MP
N balance, g/day								
Intake	19.0	18.3	19.1	18.3	0.675	0.484	0.408	0.210
Retention	9.72	9.30	9.57	9.45	0.518	0.585	0.877	0.058
N Retention/$LW^{\wedge 0.75}$	0.89	0.85	0.87	0.87	0.041	0.561	0.978	0.097
Live weight, kg								
Initial	21.3	21.3	21.6	21.0	0.212	0.789	0.088	0.002
Final	27.0	26.8	27.2	26.6	0.489	0.762	0.352	0.064
Daily gain, g	61.3	55.4	58.4	58.3	3.584	0.275	0.980	0.959
DM conversion	7.60	8.13	7.75	7.98	0.196	0.090	0.424	0.310

The ratio of methane to carbon dioxide in the breath of the sheep

Feeding potassium nitrate rather than urea decreased the ratio of methane to carbon dioxide in the eructed air from the sheep (Table 10; Figure 1). There was a tendency (P=0.18)for methane production to be reduced by supplementation with Mangosteen peel (Figure 2).

Table 10. Concentration of methane and carbon dioxide in the outside air and in the eructed breath of sheep fed a forage-based diet with potassium nitrate or urea and with or without Mangosteen peel (MP); ratios of methane to carbon dioxide are calculated according to the proposal of Madsen et al (2010)

	K-nitrate	Urea	P	With MP	Without MP	P	SEM
Air							
CO2	414	414		414	414		
CH4	2.31	2.31		2.31	2.31		
Animal							
CO2	755	611		698	668		70.5
CH4	30.8	46.5		28.6	48.7		4.63
CH4/CO2#	0.100	0.257	0.017	0.140	0.217	0.180	0.037

#Ratio CH_4/CO_2 = (CH_4animal– CH_4 air)/(CO_2animal-CO_2air), where the values in animal breath and air are in ppm

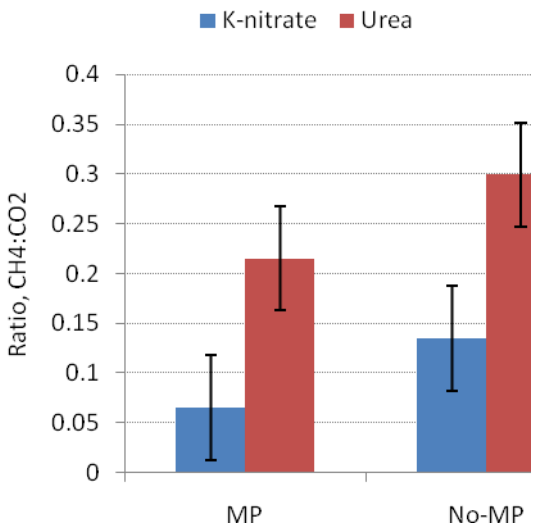

Figure 1. Effect of potassium nitrate compared with urea on the ratio of methane to carbon dioxide in eructed breath of sheep fed a forage-based diet with (MP) and without (No-MP) mangosteen peel

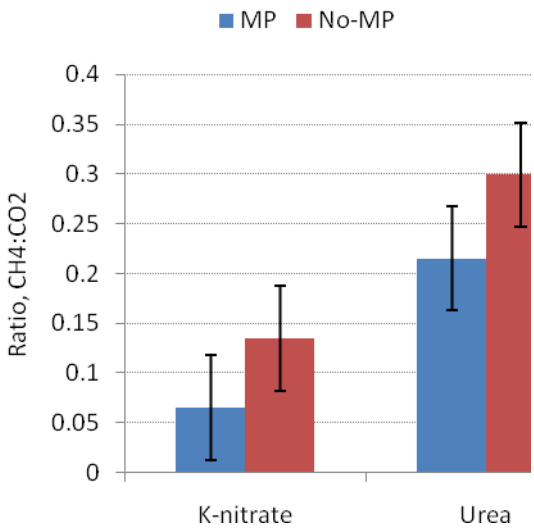

Figure 2. Effect of mangosteen peel on the ratio of methane to carbon dioxide in eructed breath of sheep fed a forage-based diet with potassium nitrate or urea

The reduction in methane production by feeding a nitrate salt instead of urea is in agreement with many reports in sheep (Nolan et al 2010; Van Zijderveld et al 2010a), goats (Nguyen Ngoc Anh et al 2010) and cattle (Van Zijderveld et al 2010b). The lack of response in growth performance, despite the reduction in methane, is also in agreement with the results reported by the above researchers.

Conclusions

Feeding potassium nitrate rather than urea decreased the ratio of methane to carbon dioxide in the eructed air from the sheep. There was a tendency (P=0.18) for methane production to be reduced by supplementation with Mangosteen peel.
There was no effect of the NPN source, nor of the supplementation with Mangosteen peel, on apparent digestibility, N retention, and growth performance.

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General Conclusions and Recommendations

Supplementation of substrates of molasses and forages with calcium nitrate, potassium nitrate and mangosteen peel resulted in reduced methane production in *in vitro* incubations.

Supplementation of basal diets of molasses and forages with potassium nitrate rather than urea had no effect on growth performance of sheep but resulted in reduced methane production. There was no effect on sheep growth performance but a tendency (P=0.18) for methane production to be reducedfrom supplementation with mangosteen peel

Further studies need to be conducted to investigate the relationships between methane production in eructed gases and growth performance in sheep suplemented with nitrate salts and/or mangosteen peel in order to relate reduction in methane emissions with animal performance.