ອິດທິພົນຂອງແກບເຜົາທີ່ນຳໃຊ້ເປັນອົງປະກອບຂອງອາຫານສັດຄ້ຽວເອື້ອງເພື່ອຫຼຸດປະລິມານທາດເມຕານ

Assessment of the influence of biochar on In vitro rumen for mitigation of methane emission

ບຸນທະວີ ວົງຄຳຈັນ[1] *ແລະ ບົວລີ ສະພັງທອງ*[2]
Bounthavy Vongkhamchanh[1] ***and Boualy Saphungthong***[2]

Abstract

The methane emissions from enteric fermentation in herbivorous animals, especially ruminants, are considered a major source of greenhouse gases. This study aimed to evaluate the effect of biochar on methane production in an *in vitro* rumen incubation in which rice straw and cassava leaf meal were used as substrate. Urea was used as sources of NPN and protein. Gas production and methane in the gas were measured over successive 6h intervals for 24h, after which the residual dry matter (DM) in the substrate was determined by filtration. During the fermentation intervals (18 – 24 h) there were significantly differences in gas production between two substrates, rice straw (276.67 ml) and cassava leaf meal (515 ml) ($p<0.05$). The levels of biochar (0%, 1% and 2%) were no affect in gas production of the substrates, in contrast biochar can be reduced methane as well as 21.292, 20.417 and 19.667 % respectively ($p<0.05$) but between 1% and 2% of biochar were not showed any difference in decreasing the methane production. The cassava leaf meal (78.479 %) was digested significantly higher than rice straw (70.139 %). Biochar did not affect the proportion of DM mineralized and digestibility after 24h, but reduced the production of methane per unit DM mineralized.

Keywords: Greenhouse gases, methane production, biochar.

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[1] Department of Livestock, Faculty of Agriculture and Forestry at Champasakt University.

Corresponding author: Tel. 020 22417209, E-mail address: vongkhamchanhd@yahoo.com

[2] Department of Livestock, Faculty of Agriculture and Forestry at Champasakt University.

Corresponding author: Tel. 020 22417209, E-mail address: vongkhamchanhd@yahoo.com

ບົດຄັດຫຍໍ້

ທາດເມເທນແມ່ນທາດໜຶ່ງທີ່ສົ່ງຜົນຕໍ່ການເກີດສະພາບໂລກຮ້ອນ ໂດຍການເຮັດໃຫ້ອຸ່ນຫະພູມຂອງໂລກເພີ່ມຂຶ້ນ, ເຊິ່ງທາດດັ່ງກ່າວສ່ວນຫລາຍແມ່ນເກີດຈາກສັດກິນພືດ ໂດຍສະເພາະແມ່ນສັດຄ້ຽວເອື້ອງ. ດັ່ງນັ້ນ ການສຶກສາໃນຄັ້ງນີ້ ແມ່ນມີຈຸດປະສົ່ງ ເພື່ອທົດສອບການນຳໃຊ້ຂີ້ແກບເຜົາ (Biochar) ເປັນອົງປະກອບຂອງອາຫານສັດ ຕໍ່ກັບການຜະລິດທາດເມເທນໂດຍການຈຳລອງກະເພາະໝັກຂອງສັດຄ້ຽວເອື້ອງ, ເຊິ່ງເຟືອງເຂົ້າ ແລະ ໃບມັນຕົ້ນແຫ້ງແມ່ນນຳໃຊ້ເປັນອາຫານທົດສອບ, ປຸ໋ຍຢູເຣຍ ແມ່ນນຳໃຊ້ເປັນແຫລ່ງໂປຣຕີນ. ວິທີການແມ່ນໄດ້ວັດແທກປະລິມານແກສລວມ ແລະ ປະລິມານທາດເມເທນ ທີ່ຜະລິດອອກມາຈາກກະເພາະໝັກທຽມໃນຊ່ວງ 0-6, 6-12, 12-18 ແລະ 18-24 ຊົ່ວໂມງ, ຫລັງຈາກການໝັກ 24 ຊົ່ວໂມງ ສິ່ງທົດລອງທັງໝົດຈະໄດ້ຕອງຜ່ານຜ້າຂາວບາງເພື່ອຊອກຫາເປີເຊັນການຍ່ອຍໄດ້. ຜົນການທົດລອງສະແດງໃຫ້ເຫັນວ່າ ລະຫວ່າງອາຫານສອງຊະນິດຄື: ເຟືອງເຂົ້າ (276.67 ml) ແລະ ໃບມັນຕົ້ນແຫ້ງ (515 ml) ແມ່ນມີການຜະລິດແກສລວມທີ່ແຕກຕ່າງກັນ ($p<0.05$). ການນຳໃຊ້ແກບເຜົາທີ່ລະດັບແຕກຕ່າງກັນຄື: 0%, 1% ແລະ 2% ແມ່ນບໍ່ມີຜົນຫຍັງຕໍ່ກັບການຜະລິດແກສລວມ ແຕ່ວ່າການນຳໃຊ້ແກບເຜົາແມ່ນຈະສາມາດຫລຸດຜ່ອນປະລິມານທາດເມເທນໄດ້ ($p<0.05$), ໃນນັ້ນ ແກບເຜົາສອງລະດັບຄື: 1% ແລະ 2% ແມ່ນບໍ່ມີຄວາມແຕກຕ່າງກັນທາງດ້ານສະຖິຕິໃນການຫລຸດຜ່ອນປະລິມານທາດເມເທນ. ເປີເຊັນການຍ່ອຍໄດ້ຂອງອາຫານສອງຊະນິດ ເຫັນວ່າ ໃບມັນຕົ້ນແຫ້ງ (78.479%) ມີປະສິດທິພາບການຍ່ອຍໄດ້ດີກ່ວາເຟືອງເຂົ້າ (70.139%). ສະນັ້ນຈາກການທົດລອງສາມາດສະຫລຸບໄດ້ວ່າ ການນຳໃຊ້ແກບເຜົາແມ່ນບໍ່ມີຜົນຕໍ່ປະສິດທິພາບການຍ່ອຍໄດ້ຂອງອາຫານ ແຕ່ມີຜົນຕໍ່ການຫລຸດຜ່ອນປະລິມານທາດເມເທນຫລັງຈາກການໝັກ 24 ຊົ່ວໂມງ ໂດຍການນຳໃຊ້ຮູບແບບການຈາລອງກະເພາະໝັກຂອງສັດເຄື້ອຍເອື້ອງ.

ຄຳສຳຄັນ: ປະກົດການເຮືອນແກ້ວ, ການຜະລິດທາດເມເທນ, ແກບເຜົາ

Introduction

As most greenhouse gases (GHG), with the exception of methane, have a half-life of over a hundred years, global GHG will have to peak by 2020 and drop by 75-80 per cent in the period to 2050 to limit global warming to two degrees (The Climate Group, 2008). The total GHG emissions for 2010 are estimated to have increased by more than 6 per cent, and for 2011 are estimated to have increased by 3.2 per cent (The Guardian, 2011; IEA, 2011). Agriculture emissions of methane (CH_4) and nitrous oxide (N_2O), which account for 90 per cent of total agricultural GHG emissions, grew by 17 per cent in the period 1990-2005, roughly proportional, for instance, to the increase in global cereal production volume, but about three times as fast as the productivity increase in global cereal production. These GHG emissions are predicted to rise by 35-60 per cent by 2030 in response to population growth and changing diets in developing countries, especially in response to greater consumption of ruminant meats and dairy products, as well as the future spread of industrial and factory farming, particularly in developing countries (IPCC, 2007).

The methane emissions from enteric fermentation in herbivorous animals, especially ruminants, are considered a major source of greenhouse gases (Stavi and Lal, 2013). Therefore, much research has been designed to investigate the mitigation of methane emissions. In an earlier report from our laboratory we showed that biochar derived from rice husks reduced methane production in an *in vitro* incubation with rumen fluid and a substrate of cassava root meal and cassava leaf meal supplemented with urea or potassium nitrate as the major fermentable N source (Leng *et al*., 2012).

The aims of this experiment were evaluated the efficacies of biochar that apply as an ingredient, the rice straw and cassava leaf meal were used as a substrate to mitigate the methane production from ruminants on using *In vitro* rumen techniques.

Materials and methods

The experiment was performed in the laboratory of Faculty of Natural Science, Champasack University. Biochar was used as a supplemented feed for reducing methane emission from in vitro rumen.

Location and duration

The experiment was conducted in the laboratory of the Faculty of Agriculture and Forestry, and Faculty of Natural resource, Champasack University, Champasak province, Lao PDR, from September, 2015 to May, 2016.

Treatments and experimental design

The experiment was designed as a 2*3 factorial in a completely randomized design (CRD) with 4 replications. The factors are:

Substrates

- RS: Rice straw
- DC: Dried cassava foliage

Source of biochar

- BIO: Biochar 1% of the DM in the substrate
- BIO: Biochar 2% of the DM in the substrate

- NOBIO: No biochar

Biochar Cassava foliage

Rice straw

Figure 1. The additive and substrates

The in vitro system

The experiment was used a simple way to determine the gas production by using recycled water bottles. A hole was made in the lid of each bottle and bottles were connected by a plastic tube through the hole in order to transfer gas during incubation. The second bottle that was suspended in water, was calibrated in steps of 40 ml indicating the amount of gas production by displacement. The gas collection bottle was then suspended in a large bottle filled with water. Finally, after adding buffer solution and rumen fluid to samples of the substrate in each bottle and filling each bottle with carbon dioxide, clay was used to cover each lid to prevent gas leaks.

Experimental procedure

The dry cassava foliage and the rice straw was chopped into small pieces around 1-2 cm in length, then ground to pass through a 1 mm sieve. The rice husks was pyrolysed to produce biochar, which was used as a supplement in the *in vitro* incubation.

Representative samples of the substrates (12 g DM includes substrate, protein source) were put in an incubation bottle to which was added 0.96 liters of buffer solution and 240 ml of rumen fluid (obtained from slaughter house), prior to filling each bottle with carbon dioxide. The bottles were incubated at 38 ^{0}C in a water bath for 24 hours.

Table 1. Ingredients of the buffer solution

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

Source: Tilly and Terry (1963).

Data collection and measurements

During the incubation the gas volume was recorded at 6, 12, 18 and 24 hours. After each time interval, the methane concentration in the gas was measured with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK). At the end of the incubation, the residual DM and N in the incubation bottle was measured to determine mineralization of the DM and of the nitrogen compounds.

Chemical analyses

Samples of fresh cassava foliage and dry cassava foliage was analysed for DM, Ash and total N according to AOAC (1990) methods.

Statistical analysis

The data will be analyzed by the General Linear Model (GLM) option in the ANOVA program of the Minitab Software (version 14.0). Sources of variation in the model are: Dried Cassava foliage (DC), biochar (BIO), interaction CF*BIO and error.

Results and Discussion

In this study, the level of biochar (0, 1 and 2%) was tested to mitigate methane emission in *In vitro* rumen with two substrates, dry cassava leaf and rice straw.

Table 2. Chemical composition for feeds

Feed	As % of DM

	DM	CP	CF	Ash
Cassava leaf	92.9	21.6	14.5	6.22
Rice straw	93.6	3.4	70.2	14.2

DM: Dry matter; CP: Crude protein; CF: Crude fibre

All intervals of incubation, the biochar was not affected in gas production (table 3), but the gas production from cassava leaf was significantly higher than rice straw ($p<0.05$). The digestibility by *in vitro* rumen was indicated that the cassava leaf higher than rice straw as much as 78.48 and 70.14 % respectively but biochar did not show any effect on digestibility. 18 – 24 h incubation intervals, biochar 1% and 2% can be reduced percentage of methane emission when compared with biochar 0%. The total DM mineralized after 24h fermentation was not affected by both substrates, cassava leaf and rice straw but biochar at 2 % was displayed the total DM mineralized higher than 0% and 1% of biochar ($p<0.05$).

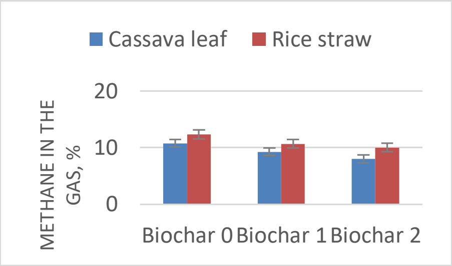
Percentage of methane in cassava leaf was lower than rice straw may due to the levels of hydrocyanic acid (HCN) precursors that contents in cassava leaf it is related to the level of methane production (Bui Huy Nhu Phuc *et al*., 2001). Some experiment was used dry cassava leaf as a substrate found it was manifested in reduced production of methane (Phuong et al 2012). The occurrence of reduced production of methane due to the toxic effect of HCN on methanogens (Smith *et al.,* 1985; Rojas *et al*., 1999).

When biochar was added as ingredient of feed with cassava root meal as substrate, and nitrate salts or urea as sources of NPN, the *In vitro* rumen incubation was used for performing the experiment. The result showed biochar reduced percentage of methane, the reason may be that the biochar had been exposed to lower temperatures during carbonization, which would have reduced the surface area per unit weight. This would have resulted in a lower capacity to adsorb nutrients and form habitat for syntrophic microbial communities in the biofilm that is believed to be a determining factor in facilitating microbial fermentation of feed organic matter (Leng *et al*., 2012). Leng et al (2013) was evaluated the efficacies of different sources of biochar were bentonite clay powder from Australia and Vietnam, two commercial samples of biochar from Australia (Bio-AL and Bio-AM), two biochar samples from Lao PDR (Bio-LP1 and Bio-LP2), it was incubated with cassava root, cassava leaf and urea. The results showed that biochar from Laos that produced by carbonization of rice husks in an updraft gasifier stove can improve the capacities in decreasing methane production (from 8 to 14%) when the incubation time was extended to 48 h.

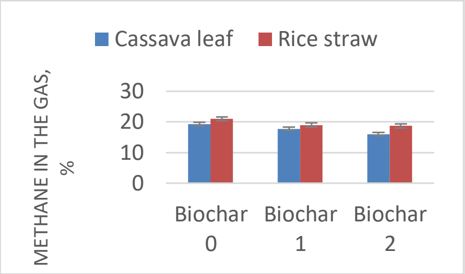
Table 3. Mean values of gas production, percent of methane in the gas, methane (ml), DM mineralized, and methane per unit of DM mineralized for different processing of cassava root and supplementation with biochar

	CM	RT	SEM	Prob.	B0	B1	B2	SEM	Prob.
0-6hours									
Gas production, ml	447.50^{a}	311.10^{b}	9.26	<0.001	374.20	385.00	378.80	12.25	0.823
Methane, %	9.333^{b}	11^{a}	0.39	0.01	11.542^{a}	9.958^{b}	9^{b}	0.51	0.010
Methane, ml	41.34^{a}	34.23^{b}	1.26	0.005	42.58^{a}	37.60^{ab}	33.20^{b}	1.65	0.002
6-12hours									
Gas production, ml	677.5^{a}	573.3^{b}	17.529	<0.001	595.8	620.4	660	23.19	0.177
Methane, %	17.667^{b}	19.556^{a}	0.3177	0.001	20.125^{a}	18.375^{b}	17.333^{b}	0.42	0.001
Methane, ml	119.13	111.9	3.652	0.2143	119.44	113.59	113.52	4.78	0.619
12-18hours									
Gas production, ml	809.2^{a}	680^{b}	19.827	<0.001	732.1	748.8	752.9	26.23	0.840
Methane, %	19.417^{b}	21.333^{a}	0.3184	0.001	21.292^{a}	20.042^{ab}	19.792^{b}	0.42	0.051
Methane, ml	157.22	145.09	5.3419	0.1577	155.21	149.21	149.05	6.99	0.785
18-24hours									
Gas production, ml	515^{a}	276.7^{b}	13.961	<0.001	411.2	378.3	397.9	18.47	0.466
Methane, %	19.583^{b}	21.333^{a}	0.2555	<0.001	21.292^{a}	20.417^{ab}	19.667^{b}	0.34	0.014
Methane, ml	100.85^{a}	59.27^{b}	3.3089	<0.001	86.429	76.367	77.379	4.33	0.234
Digested, %	78.48^{a}	70.14^{b}	0.4713	<0.001	73.97^{a}	73.45^{ab}	75.51^{b}	0.62	0.083
Methane, ml/g DM substrate mineralized	44.46	41.7	1.0647	0.109	45.41^{a}	42.74^{ab}	41.08^{b}	1.41	0.124

CM: Cassava leaf meal, RT: Rice straw, B0: Biochar 0%, B1: Biochar 1%, B2: Biochar 2%
abc Means in same row without common superscript differ at p<0.05



0-6h



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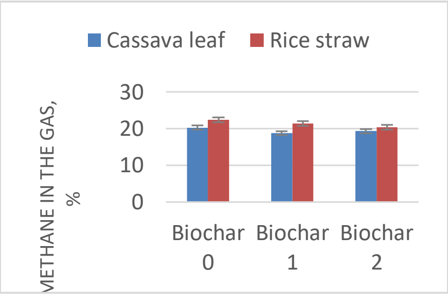

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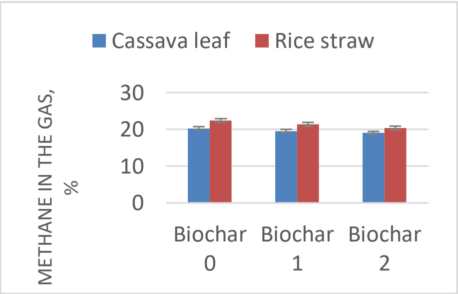
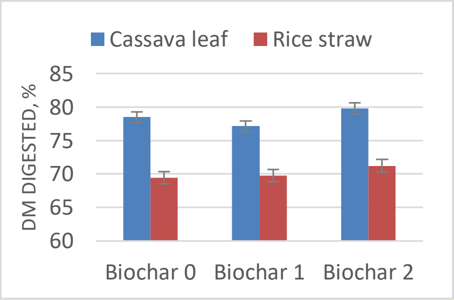


18-24h

Figure 2 Methane concentration in the gas was reduced when using biochar.



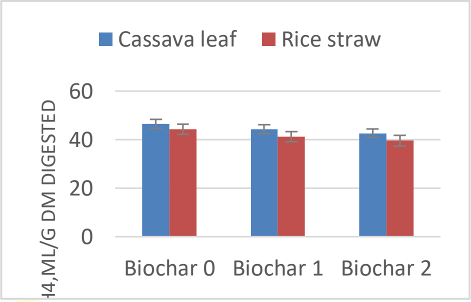


Figure 3 the effect of biochar on methane per unit substrate mineralized from cassava leaf and rice straw.

Conclusion

In an *in vitro* rumen fermentation methane percent in the gas was lowest when the cassava leaf was added as substrate. Biochar reduced methane production, but it was not affected in gas production and digestibility.

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