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Effects of different levels of biochar on methane, carbon dioxide production and digestibility of para grass (*Brachiaria mutica)* in *in vitro* incubation

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ABSTRACT

The objectivie of this study was to evaluate adding levels of biochar on methane, carbon dioxide and digestibility of para grass in in vitro incubation. The experiment was arranged in a completely randomized design with three replications of five treatments which are different levels of biochar added to para grass (Brachiaria mutica) at 0, 0.5, 1.0, 1.5, and 2.0%, namely BC0, BC0.5, BC1, BC1.5, and BC2, respectively. The incubation lasted for 72 hours with measurements of gas, methane and carbon dioxide production at 0, 6, 12, 24, 48 and 72 hours. The results showed that the total CH_4 *production (ml/g) and organic matter digestibility (%) after 72-hour incubation significantly decreased (P<0,05) among the treatments by increase of levels of biochar with the highest values for the BC0 treatment (54.8ml/g and 71.6%, respectively). It could be concluded that adding biochar to the para grass could reduce in vitro gas, methane and carbon dioxide production up to 2.0%; however, the reduction of organic matter digestibility was also found. These results supply key information for application in vivo study to reduce greenhouse emissions and increase ruminant performance.*

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1 INTRODUCTION

In recent years, many studies have reported that the addition of biochar may reduce methane emissions such as biochar reduces enteric methane and improves growth and feed conversion in local "yellow" cattle fed cassava root chips and fresh cassava foliage (Leng *el al*., 2012). Biochar and leaves from sweet or bitter cassava reduced *in vitro* gas and methane production using cassava root pulp as source of energy (Phanthavong *el al*., 2015). Biochar is a carbon-rich product that is obtained by burning biomass (such as rice husks, straw, water hyacinth, bamboo, melaleuca, wood, or leaves) in an enclosed chamber (anaerobic or less air). The purpose of this experiment was to determine effects of adding biochar on methane, carbon dioxide production and digestibility of para grass.

2 MATERIALS AND METHODS

2.1 Location of this study

The experiment was conducted at the Laboratory E205, Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University, from July to November, 2017.

2.2 Experimental design

The experiment was a completely randomized design with five treatments and three replications. The treatments were the level of 0, 0.5, 1.0, 1.5, and 2.0% of biochar added to para grass (*Brachiaria mutica*) corresponding to the BC0, BC0.5, BC1, BC1.5 and BC2 treatment.

Table 1: Ingredients in the substrate (g dry matter basis) of treatments

Feed	Treatments				
	BC0	BC0.5	BC1.0	BC1.5	BC2.0
Para grass	0.2	0.2	0.2	0.2	0.2
Biochar	0	0.001	0.002	0.003	0.004

2.3 Materials and methods

In vitro gas production was done following the procedure described by Menke *el al*. (1979). The para grass was cut into small pieces, about 1 cm of length, and then dried at 65˚C during 24 hours, then ground through 1 mm seive. Representative samples (0.2 gDM of para grass) were put into the incubation 50-ml syringes, which were added buffer solution and cattle rumen fluid, prior to filling each bottle with carbon dioxide following the method described by Menke *el al*. (1979). Then, the syringes were put in the water bath at 39ºC. Gas, CH_4 and CO_2 volumes over time (0, 6, 12, 24, 48 and 72 hours) were recorded, while the CH_4 and CO_2 concentrations were measured by the Biogas 5000 Geotechnical Instruments (UK) Ltd, England. Unfermented solids at 24, 48 and 72 hours were determined by filtering through two layers of cloth and drying at 105°C for 24 hours and ashing for 5 hours to measure the dry matter digestibility (DMD) and organic matter digestibility (OMD), respectively.The biochar used in the experiment was made from by burning rice husk with the anaerobic condition at 500ºC by Electronic furnace VMF-165, Yamada Denki Co. Ltd., Japan at College of Environment and Natural Resources, Can Tho University.

The ingredients in the substrate were analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to the standard methods of AOAC (1990), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were analyzed following procedures suggested by van Soest *el al.* (1991).

The measurements of the experiment were gas, CH_4 and CO_2 production; OMD at 0, 6, 12, 24, 48 and 72 hours. The experiment data were analyzed through ANOVA (with general linear model) and Tukey test (with Minitab 2010 software).

3 RESULTS AND DISCUSSION

3.1 Chemical composition of materials

The chemical composition of feeds was presented in Table 2.

Table 2. Chemical composition (%) of feeds used in the experiment

Feed	DM	OM	CP	EE	NFE	CF	NDF	ADF	Ash
Para grass	95.2	88.5	11.0	3.52	45.1	28.8	64.8	32.7	11.5
Biochar	99.6	64.3	2.53	1.90	2.87	57.0	62.9	64.4	35.7

DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

Table 2 showed that para grass had dry matter (DM) content (95.2%) and the crude protein (CP) content (11.0%) that were lower than those of Tran Thi Dep (2012) and Nguyen Thi Thu Hong *el al.* (2008) were 12.8% because Para grass used in the experiment was harvested in the dry season, it had a high DM content, but low CP content. Biochar had high ash with the content of 35.7%.

3.2 *In vitro* gas production

The in vitro gas production was presented in Fig. 1. In general, the total accumulated gas increased with increases in incubation time. It is also showed that the accumulated gas production was similar in different treatments from 0 to 48 hours, while it was slightly higher for the BC0 treatment from 60 to 72 hours compared to the others (Fig. 1). The BC0 treatment was not added biochar. Biochar has a porous structure on the surface and system of porous holes between 1 nm and a few thousand nm; therefore, it can adsorb and fix carbon. The addition of biochar reduced gas production (Leng el al., 2012).

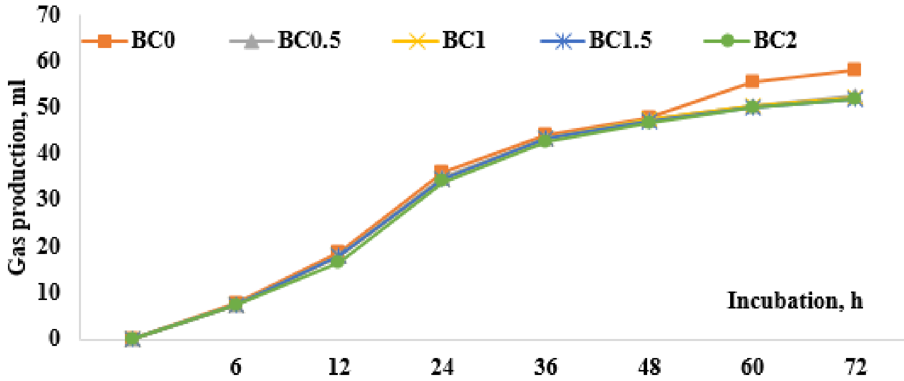

Fig. 1: In vitro gas production as affected by different levels of biochar over incubation times

In vitro CH_4 and CO_2 production and OMD was showed in Fig. 2 and Table 3.

The *In vitro* methane production of different treatments was generally similar in development patterns with the higher values for the BC0 treatment (Fig. 2) and the lower values for the BC2, particularly from 60 to 72 hours (P<0.05).

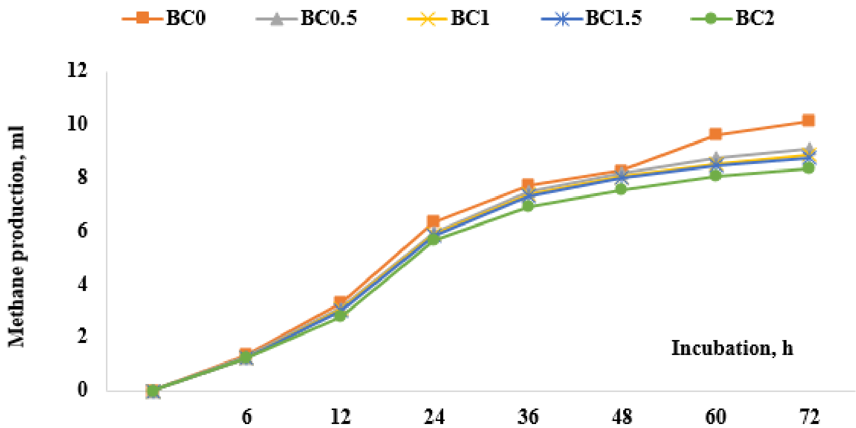

Fig. 2: In vitro methane production as affected by different levels of biochar over incubation times

Table 3: Gas, CH_4 and CO_2 production (ml), DM and OMD (%) in different treatments at 72 hours in the experiment

Item	Treatment					±SE	P
	BC0	BC0.5	BC1.0	BC1.5	BC2.0		
Gas, ml	58,2^{a}	52,6^{b}	52,4^{b}	52,0^{b}	51,8^{b}	1,233	0,001
CH_4, ml	10,1^{a}	9,08^{b}	8,88^{bc}	8,75^{bc}	8,34^{c}	0,244	0,001
CO_2, ml	31,2^{a}	28,2^{b}	27,8^{b}	27,4^{b}	26,7^{b}	0,667	0,001
DMD,%	69,5^{a}	62,6^{b}	62,2^{b}	61,5^{b}	60,5^{b}	1,239	0,001
OMD,%	71,6^{a}	65,4^{b}	63,5^{b}	63,3^{b}	62,5^{b}	1,295	0,001
Gas, ml/gOM	316^{a}	285^{b}	284^{b}	282^{b}	281^{b}	6,685	0,001
Gas, ml/gDOM	441	436	448	446	450	8,716	0,329
CH_4, ml/gOM	54,8^{a}	49,3^{b}	48,2^{bc}	47,5^{bc}	45,2^{c}	1,324	0,001
CH_4, ml/gDOM	76,5	75,4	75,9	75,0	72.4	1,563	0,069
CO_2, ml/gOM	169^{a}	153^{b}	151^{b}	149^{b}	145^{b}	3,617	0,001
CO_2, ml/gDOM	236	234	238	235	232	5,480	0,796

BC0, BC0.5, BC1, BC1.5 and BC2: level of 0%, 0.5%, 1%, 1.5% and 2% of biochar added Para grass. $^{a, b, c}$Means with different letters within the same rows were significantly different at the 5% level

The total gas, CH_4 and CO_2 production (ml) at 72 hours were significantly different (P<0.05) among the treatments with the lowest values for the of BC2 treatment. Similarly, the gas, CH_4 and CO_2 production (ml/gOM) were gradually decreased (P<0.05) from the BC0 to the BC2 treatment. However, the *in vitro* DM and OMD values were also reduced (P<0.05) from the BC0 to the BC2 treatment with the lowest value of OMD for the BC2 treatment (62.5%) compared to that of the BC0 treatment (71.6%). The results were higher than those (ml/gOM) reported by Nguyen Van Thu *el al.* (2016) studying on ground maize being 222 (gas) and 33.3 (CH_4). Generally, the greenhouse production and OMD were reduced when increasing the biochar levels from 0 to 2.0%. Leng *el al.* (2012) asserted that adding 1% biochar to diets reduced 11-13% of methane production; however, increasing the amount of biochar by 2% to 5% did not reduce methane production. The linear relationship of levels of biochar (%) and methane production (ml/g OM) is high, when the biochar rate increases, the methane production decreases linearly with R^2 = 0.772 with the y = - 4.19 + 53.2 (Fig. 3).

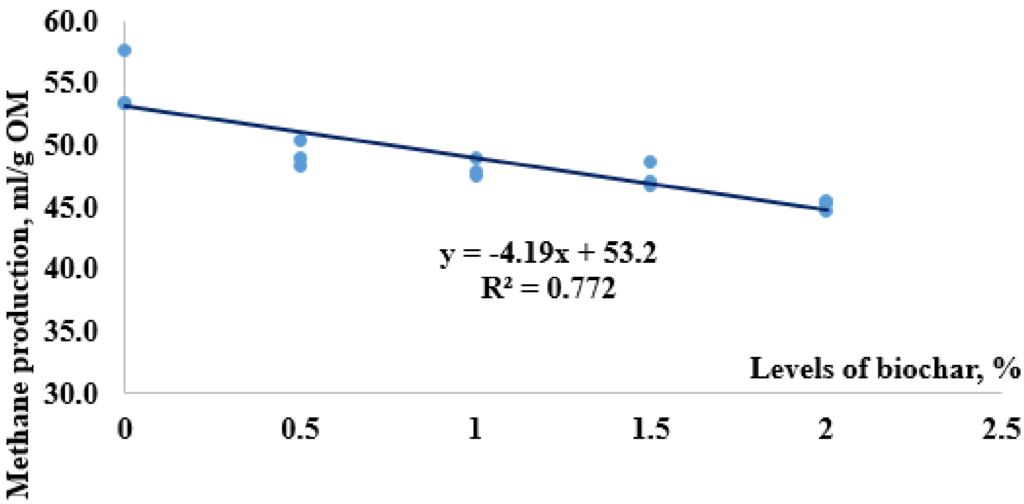

Fig 3: Linear relationship between biochar (%) and *in vitro* methane production

The accumulated gas, CH_4 and CO_2 production (ml/g DOM) in the experiment was not significantly different among the treatments at 72 hours. The results of gas and methane production (ml/g DOM) were similar to those reported at 72 hours by Nguyen Ngoc Duc An Nhu (2016) studying with only para grass (462 and 88.8, respectively) and Huynh Doan Nghich Luy (2016) studying with para grass supplemented probiotics (469 and 97.3, respectively).

4 CONCLUSION AND RECOMMENDATION

It was concluded that the *in vitro* gas, CH_4 and CO_2 production (ml/g OM) was gradually reduced by increasing levels of biochar to the substrate of para grass from 0 to 2.0%. At 72 hours, methane production (ml/g OM) was reduced from 10 to 17.5% when adding 0.5 – 2 % of biochar compared to no biochar addition. However, a gradual reduction of DM and OMD when increasing biochar levels has been found in this study. The *in vivo* experiments on ruminants with biochar in the diets should be implemented to confirm the results for a potential application.

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