IN VITRO METHANE AND CARBON DIOXIDE PRODUCTION AND ORGANIC MATTER DIGESTIBILITY AFFECTED BY PARA GRASS (*BRACHIARIA MUTICA*) REPLACEMENT TO RICE STRAW

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ABSTRACT

The aim of the experiment was to evaluate the CH_4 and CO_2 production and organic matter digestibility (OMD) by replacing Para grass to rice straw as the main substrate. This experiment was arranged in a complete randomized design *in vitro* condition with 5 treatments and 3 replicates by using the glass syringe system. The treatments included 100% rice straw (PG0), 75% rice straw + 25% Para grass (PG25), 50% rice straw + 50% Para grass (PG50), 25% rice straw + 75% Para grass (PG75) and 100% Para grass (PG100) based on DM basis. The results indicated that *in vitro* CH_4 and CO_2 production (ml/g DOM) at 72 h were significantly different (P<0.05) among the treatments and gradually increased from PG0 (35.4) to PG100 treatment (65.7). The linear relationship between CH_4 production (ml/gDOM) and Para grass replacement to rice straw was closed with the function y = 0.306x +34.1 (R^2 = 0,972). Similarly, the OM digestibility significantly different (P<0.05) among the treatments and was 42.0, 49.8, 56.8, 63.9 and 79.9% for the PG0, PG25, PG50, PG75 and PG100 treatment, respectively. It was concluded that there was an increase of *in vitro* GHG production and OMD when increasing the replacement levels of Para grass to rice straw, and the nitogen-free extract (NFE) had a possitiverelationto the methane production.

Keywords: *degradation, fermentation, local feed sources, roughages, ruminants.*

INTRODUCTION

It is indicated that from 85 to 90% methane is produced by enteric fermentation (Hindrichsen et al., 2005) and there is a great incentive to reduce methane emissions from ruminant production. Recently feeding strategies for ruminants focusing to reduce greenhouse gas and to improve their performance have been considered in priority.In Mekong delta of Vietnam Para grass (*Brachiaria mutica*) and rice straw are popularly fed buffaloes and cattle depending on the rainy or dry season. However, rice straw has more structural carbohydrates and less nutrients and digestibility compared to the Para grass. Sauvant and Giger-Reverdin (2009) indicated that ruminant fed forages rich in structural carbohydrates produce more CH_4 than those fed mixed diets containing higher levels of non-structural carbohydrates per unit of fermented material in the rumen. This is explained by the different metabolic routes used to ferment the different carbohydrates which result in different VFA profiles that yield more or less metabolic H_2 as the main substrate to produce CH_4 (Hristov et al., 2013). For feeds with higher digestibility, however, increased intake results in a depression in the amount of CH_4 produced per unit of feed consumed. Forages are the feed ingredients with the largest variability in composition and have the largest impact on diet digestibility (Yáñez-Ruiz et al., 2017).While factors, such as plant species, variety, maturity at harvest and preservation can all affect forage quality and digestibility. Thus an evaluation of greenhouse production and digestibility *in vitro* of rice strawand Para grass should be done for further *in vivo* and applied studies.

MATERIALS AND METHODS

Materials and *in vitro* gas production technique

The Para grass and rice straw was harvested about 50-60 cm from the ground, then they will be cut from 2 - 3 cm length to dry at 55°C for 48 hours and finely ground.

Representative samples (0.2 gDM of the substrate) were put into the incubation 50-ml syringes. Buffer solution and cattle rumen fluid were added, prior to filling each bottle with carbon dioxide following the method described by Menke and Steingass (1988). Then, the syringes were put in the water bath at 39°C for an incubation.

Location and time

The experiment was conducted at the laboratory of Department of Animal Science, College of Agriculture, Can Tho City from Aug, 2018 to Febuary, 2019.

Experimental design

This experiment was arranged in a complete randomized design in *vitro* condition with 5 treatments and 3 replicates. The treatments included 100% Rice straw (PG0), 75% rice straw + 25% Para grass (PG25), 50% rice straw + 50% Para grass (PG50), 25% rice straw + 75% Para grass (PG75) and 100% Para grass (PG100) based on DM basis.

Measurements taken

Chemical compositions of the substrate ingredients, which 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) were analyzed following procedures suggested by Van Soest et al. (1991).

Total gas, CH_4 and CO_2 production. Gas, CH_4 and CO_2 volumes over time (0, 3, 6, 9, 12, 24, 48 and 72 hours) were recorded and collected, while the CH_4 and CO_2 concentrations were measured by the Biogas 5000 Geotechnical Instruments (UK) Ltd, England.

Organic matter digestibility (OMD) at 24, 48 and 72 hours. Unfermented solids at 72 hours was 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.

Statistical analysis

The experiment data were calculated by Excel software and statistically analyzed by using ANOVA with the general linear model (GLM) following the complete randomized design and the Tukey test was used for a comparison of two treatments (Minitab, 2010).

RESULTS AND DISCUSSION

Chemical compositions of substrate ingredients

The chemical compositions of Para grass and rice straw were presented in the Table 1.

Table 1. 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	40.4	28.8	64.8	32.7	11.5
Rice straw	89.9	84.2	4.1	1.97	36.4	31.6	65.1	43.7	15.8

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

Table 1 showed that dry matter (DM) content of Para grass was 95.2% and was similar to that reported by Nguyen Ngoc Duc An Nhu (2016) when studyingeffects of carbohydrates and crude protein on *in vitro* gas production with substrates of Para grass being 94.8%. Crude protein (CP) content of Para grass was 11.0%, while this was much lower value for rice straw (4.10%). They were slightly lower than those reported by Le Thi Ngoc Huyen et al. (2010) with CP of Para grass and rice straw being 11.6 and 4.70%, respectively. However NDF content of Para grass (64.8%) was similar to that of rice straw (65.1%). They were lower than those presented by Doan Huu Luc et al. (2009) with NDF content of Para grass and rice straw being 69.1 and 72.3%. Rice straw had ADF content of 43.7%, which was higher than that of Para grass (32.7%). Particularly NFE of Para grass (40.4) was much higher than that of rice straw.

The nutrient amounts of the treatments of the experiment after calculation were described in Table 2.

Table 2. The nutrient amounts (g) contained in different treatments of the experiment

Nutrient	**Treatments**				
	PG0	***PG25***	***PG50***	***PG75***	***PG100***
DM	0.180	0.182	0.185	0.188	0.190
OM	0.168	0.171	0.173	0.175	0.177
CP	0.008	0.012	0.015	0.019	0.022
EE	0.004	0.005	0.005	0.006	0.007
NFE	0.073	0.075	0.077	0.079	0.081
CF	0.063	0.062	0.060	0.059	0.058
NDF	0.130	0.130	0.130	0.130	0.130
ADF	0.087	0.082	0.076	0.071	0.065
Ash	0.032	0.029	0.027	0.025	0.023

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

In Table 2 indicated that when increasing the Para grass replacement levels to rice straw the DM, OM, CP, EE, and NFE were gradually enhanced from the PG0 to PG100 treatment, while there was a reduction of NDF, ADF, CF and ash amount. It was clearly showed that the amount of NFE and CP containing in the PG replacement treatments, which increased following the gradual augment of the PG.

In vitro gas, CH_4 and CO_2 production over incubation times

The *in vitro* gas,CH_4 and CO_2 production were demonstrated in the Fig. 1, 2 and 3.

In general the total gas, CH_4 and CO_2 production increased with the increasing incubation time. It was also showed that the gas, CH_4 and CO_2 production from 0 to 72 h were gradually high from PG0 to PG100 treatment. Thus they enhanced with increasing Para grass levels to the rice straw from 0 – 100%.

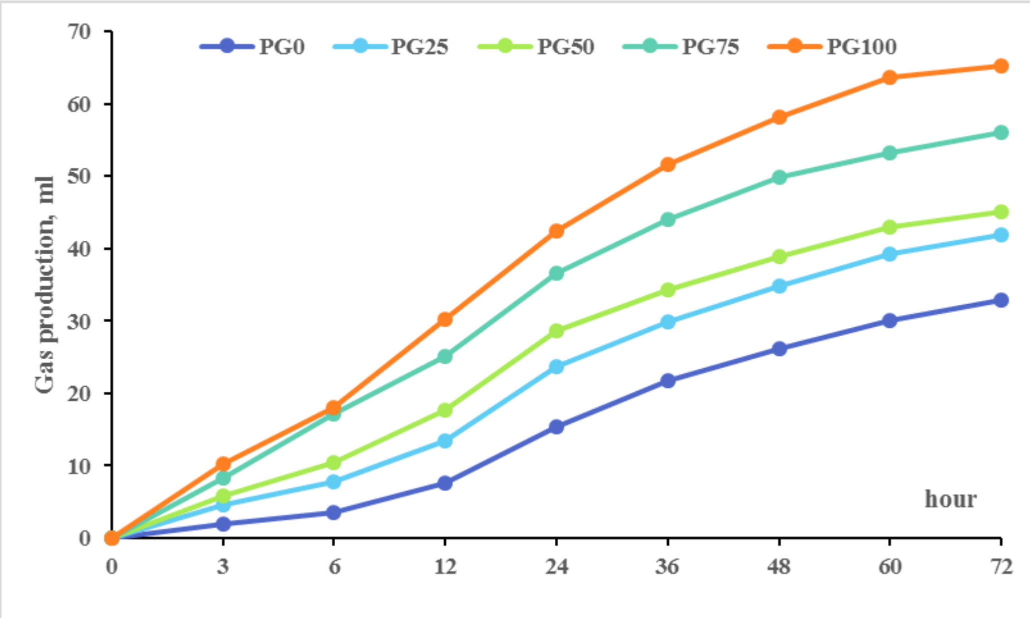

Fig 1. *In vitro* gas production of the different treatments over incubation times

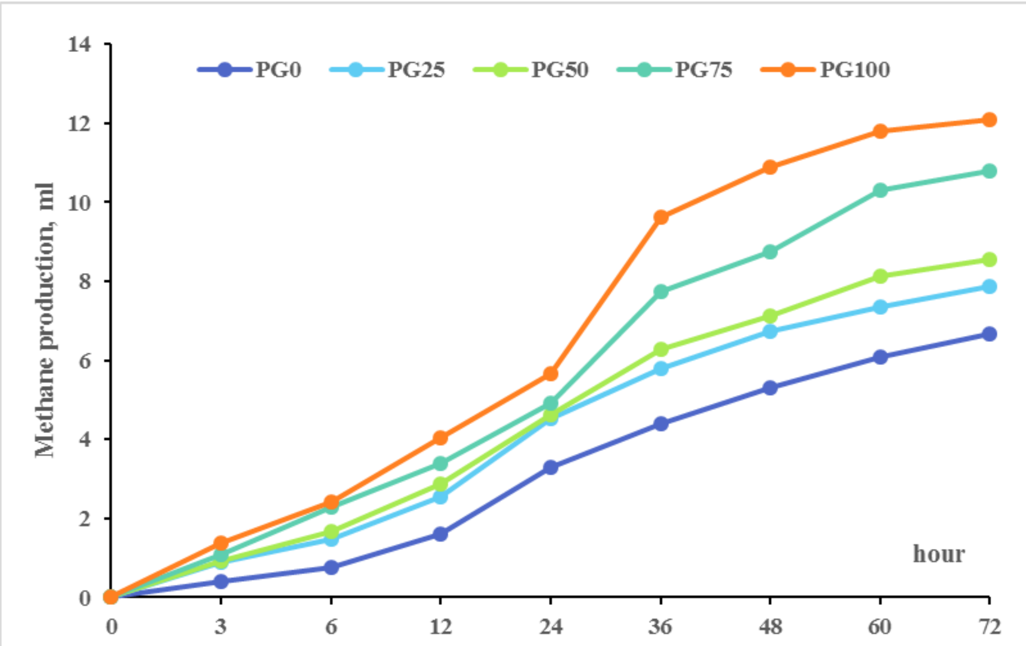

Fig 2. *In vitro* methane production of different treatments over incubation times

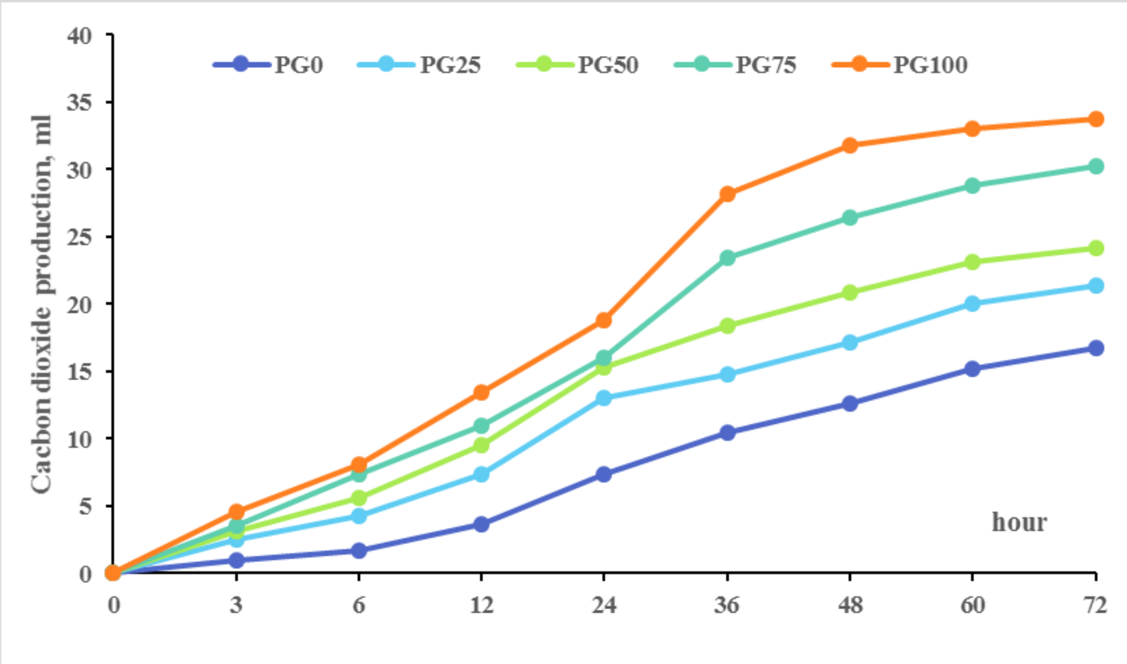

Fig 3. *In vitro* carbon dioxide production of different treatments over incubation times

***In vitro* gas, CH_4 and CO_2 production, and OM digestibility(OMD) at 24 hours**

The *in vitro* gas, CH_4 and CO_2 production, and OM digestibility at 24 h was showed in Table 3.

Table 3. Gas, CH_4 and CO_2 production and OMD at 24 hours in different treatments

Item	Treatments					SE	P
	PG0	*PG25*	*PG50*	*PG75*	*PG100*		
Gas, ml	15.4^e	23.7^d	28.6^c	36.6^b	42.4^a	0.331	0.001
CH_4, ml	3.29^c	4.52^b	4.63^b	4.92^b	5.65^a	0.241	0.001
CO_2, ml	7.34^d	13.0^c	15.3^b	16.0^b	18.8^a	0.477	0.001
OMD, %	17.4^e	41.9^d	49.5^c	53.7^b	67.7^a	0.202	0.001
Gas, ml/g OM	81.7^e	127^d	153^c	198^b	230^a	1.78	0.001
CH_4, ml/g OM	17.5^c	24.1^b	24.8^b	26.6^b	30.6^a	1.30	0.001
CO_2, ml/g OM	39.0^d	69.5^c	82.4^b	86.4^b	102^a	2.58	0.001

100% Rice straw (PG0), 75% rice straw + 25% Para grass (PG25), 50% rice straw + 50% Para grass (PG50), 25% rice straw + 75% Para grass (PG75) and 100% Para grass (PG100) OMD: organic matter digestibility. $^{a, b, c,d}$ Means with different letters within the same rows were significantly different at the 5% level(P<0,05).

Table 2 showed that gas, CH_4 and CO_2 production (ml) at 24 h was significantly different among the treatments ($P<0.05$) at 24 h. The gas, CH_4 and CO_2 production (ml) were the highest values for the PG100 treatment (42.4, 5.65 and 18.8 ml, respectively) and the lowest values for the PG0 treatment (15.4, 3.29 and 7.34 ml, respectively). The values were higher than those reported by Doan Huu Luc et al. (2009) studying *In vitro* gas production at 24 h from rice straw and soybean meal was 10.2 ml. The OMD (%) was also significantly different among the treatments (P<0.05) with lowest value for the PG0 treatment with 100% rice straw (17.4%), while the PG25, PG50, PG75 and PG100 treatments were 41.9, 49.5, 53.7% and 67.7%. Similarly the gas, CH_4 and CO_2 production (ml/g OM) were significantly different among the treatments (P<0.05) and they increased by the enhancing the levels of Para grass replacement.

***In vitro* total gas, CH_4 and CO_2 production and OM digestibility at 48 hours**

The *in vitro* gas, CH_4 and CO_2 production, and OM digestibility at 48 h were showed in Table 4.

Table 4. Gas, CH_4 and CO_2 production and OM digestibility (OMD) at 48 hours in different treatments

Item	PG0	PG25	PG50	PG75	PG100	SE	P
Gas, ml	26.1^{e}	34.8^{d}	38.9^{c}	49.8^{b}	58.2^{a}	0.630	0.001
CH_4, ml	5.30^{d}	6.72^{c}	7.14^{c}	8.75^{b}	10.9^{a}	0.316	0.001
CO_2, ml	12.6^{e}	17.2^{d}	20.9^{c}	26.4^{b}	31.8^{a}	1.014	0.001
OMD, %	42.0^{e}	49.8^{d}	56.8^{c}	63.9^{b}	79.9^{a}	0.364	0.001
Gas, ml/g OM	139^{e}	186^{d}	209^{c}	269^{b}	316^{a}	3.39	0.001
CH_4, ml/g OM	28.2^{d}	35.9^{c}	38.3^{c}	47.2^{b}	59.0^{a}	1.70	0.001
CO_2, ml/g OM	66.8^{e}	92.1^{d}	112^{c}	142^{b}	173^{a}	5.47	0.001

100% Rice straw (PG0), 75% rice straw + 25% Para grass (PG25), 50% rice straw + 50% Para grass (PG50), 25% rice straw + 75% Para grass (PG75) and 100% Para grass (PG100) OMD: organic matter digestibility. *[a, b, c,d] Means with different letters within the same rows were significantly different at the 5% level (P<0.05).*

Table 4 indicated that gas, CH_4 and CO_2 production (ml) were significantly different among the treatments (P<0.05) at 48h. The gas, CH_4 and CO_2 production (ml) were the lower values for the PG0 treatment (26.1, 5.30 and 12.6 ml, respectively) and the higher values for the PG100 treatment (58.2, 10.9 and 31.8 ml, respectively), Vo Duy Thanh et al. (2012) reported that when studying 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 substrates,CH_4 production was 19.3 ml at 48h. Beside OMD (%) was significantly different among the treatments (P<0.05), the lowest value for PG0 treatment (42.0%), other values of treatments of PG25, P50, PG75 and PG100 treatments were 49.8, 56.8, 63.9%) and 79.9%. The values of gas, CH_4 and CO_2 production (ml/g OM and DOM) was also significantly different among the treatments (P<0.05), while the lowest value for PG0 treatment (139, 28.2 and 66.8 ml/g OM, respectively) and the highest values for PG100 treatment (316, 59.0 and 173 ml/g OM, respectively).

***In vitro* total gas, CH_4, CO_2 production and OM digestibility at 72 hours**

The accumulated *in vitro* gas, CH_4 and CO_2 production, and OM digestibility at 72 h were presented in Table 5.

Table 5. Gas, CH_4 and CO_2 production and OM digestibility at 72 hours

Item	PG0	PG25	PG50	PG75	PG100	SE	P
Gas, ml	32.9^{e}	41.9^{d}	45.1^{c}	56.0^{b}	65.3^{a}	0.873	0.001
CH_4, ml	6.67^{d}	7.86^{c}	8.54^{c}	10.8^{b}	12.1^{a}	0.408	0.001
CO_2, ml	16.7^{e}	21.4^{d}	24.2^{c}	30.2^{b}	33.8^{a}	0.859	0.001
OMD, %	53.1^{e}	59.6^{d}	65.8^{c}	72.6^{b}	88.0^{a}	0.73	0.001
Gas, ml/g OM	175^{e}	224^{d}	242^{c}	302^{b}	354^{a}	4.68	0.001
CH_4, ml/g OM	35.4^{d}	42.0^{c}	45.8^{c}	58.2^{b}	65.7^{a}	2.19	0.001
CO_2, ml/g OM	88.6^{e}	114^{d}	130^{c}	163^{b}	184^{a}	4.59	0.001
Gas, ml/g DOM	329^{c}	375^{b}	368^{b}	416^{a}	403^{a}	8.14	0.001
CH_4, ml/g DOM	66.7^{b}	69.6^{b}	70.4^{b}	74.6^{ab}	80.1^{a}	3.11	0.003
CO_2, ml/g DOM	167^{d}	191^{c}	197^{bc}	208^{ab}	225^{a}	6.40	0.001

100% Rice straw (PG0), 75% rice straw + 25% Para grass (PG25), 50% rice straw + 50% Para grass (PG50), 25% rice straw +75% Para grass (PG75) and 100% Para grass (PG100) OMD: organic matter digestibility.$^{a, b, c,d}$ *Means with different letters within the same rows were significantly different at the 5% level(P<0.05).*

The accumulated total gas, CH_4 and CO_2 production (ml) were significantly different among the treatments (P<0.05) at 72h. The total gas, CH_4 and CO_2 production (ml) were the lowest values of PG0 treatment (32.9, 6.67 and 16.7 ml, respectively) and the highest values of PG100 treatment (65.3, 12.1 and 33.8 ml, respectively). In this study the total gas (ml) produced was rather higher than that presented by Doan Huu Luc et al. (2009) being 58.0 ml. Beside, OMD values (%) at 72 h were significantly different among the treatments (P<0.05), they were 53.1, 59.6, 65.8, 72.6 and 88.0 % for the PG25, P50, PG 75 and PG100 treatment, respectively. The gas, CH_4 and CO_2 (ml/g OM and DOM) were similar in production patterns of the above (calculated by ml). It was indicated that the linear relationship between methane production at 72h (ml/g OM) [y] and levels of Para grass replacement (%) [x] and was very closed with $R^2 = 0.972$ of the function $y = 0.306x + 34.1$.

In summary, the results of the present study demonstrated that when augmenting the Para grass levels to replace rice straw as the main substrate from 0 to 100%,the greenhouse gas production gradually increased. Sauvant and Giger-Reverdin (2009) reported that ruminant fed forages rich in structural carbohydrates produce more CH_4 than those fed mixed diets containing higher levels of non-structural carbohydrates per unit of fermented material in the rumen. Then Yáñez-Ruiz et al. (2017) indicated that feeds with higher digestibility increased intake results in a depression in the amount of CH_4 produced per unit of feed consumed. However, in the present study rice straw is higher in CF and ADF (structural carbohydrates), which induced the lower digestibility, and then produced less methane compared to Para grass. In other words, Para grass with higher levels of non-structural carbohydrates and digestibility; however it produced more methane volume (Table 3, 4 and 5). Table 2 was clearly demonstrated that the PG replacement treatments had the higher amount of NFE and CP. Lee et al. (2003) reported that it seemed possible to predict methane production potential from nutritional composition of the ingredient for their effective application on for mulating less methane emitting rations and methane production from each feed ingredient decreased within creasing amount of crude fiber (CF) and ether extract (EE), where as positive relationship was noted with the concentrations of NFE. Shibata et al. (1992) from the multiple regression analyses relating CH_4 production to various nutrient intakes also suggested that NFE had positive correlation with methane production. Similarly Nguyen Binh Truong and Nguyen Van Thu (2020) in a multi-regression analysis of CH_4 production and the nutritional composition of different feeds concluded that there was the first significant affect of NFE on the *in vitro* CH_4 production and Lee et al. (2003) also demonstrated that NFE was the most important factor in methane production different from other results.

CONCLUSION AND RECOMMENDATION

It was concluded that when enhancing replacement levels of Para grass to rice straw as the substrate, *in vitro* gas, CH_4 and CO_2 production and organic matter digestibility gradually increased from PG0 to PG100 treatments. At 72 h after incubation the methane production and the replacement levels of Para grass was linearly correlated with thefunction $y = 0.306x +34.1$ ($R^2 = 0.972$). There was an important affect of NFE on the *in vitro* methane production, which was found in the present study.

It is necessary to confirm the results by *in vivo* and performance studies for an approriate application to mitigate the enteric greenhouse gas production.

ACKOWLEDGEMENT

Chemicals, equipment and Lab works of this research is provided by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan. The Authors also thank Dept of Animal Sciences of College of Agriculrure, Can Tho University for facilitating the equipments using and Laboratory works of the experiments.

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Received date: 25/8/2020

Submitted date: 04/9/2020

Acceptance date: 21/10/2020

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