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EFFECTS OF BIOCHAR PRODUCED FROM TROPICAL RICE HUSK AND PEANUT SHELL AT DIFFERENT PROCESSING TEMPERATURES ON *IN VITRO* RUMEN FERMENTATION AND METHANE PRODUCTION

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ABSTRACT

The aim of this study was to investigate the effects of biochar produced from tropical biomass resources at different processing temperatures on methane production and rumen fermentation *in vitro*. Two available tropical biomass resources of rice husk and peanut shell were used for pyrolysis at three temperature levels of 300, 500 and 700ºC. Biochar was supplemented at 3% in diets of dry matter basis. *In vitro* fermentation characteristics and methane production were measured at 4, 24 and 48h after incubation. Results showed that there were no significant differences in terms of (i) gas and methane production, (ii) dry matter and organic matter digestibility (iii) pH and NH_3-N concentration between diets supplemented either rice husk or peanut shell drived biochar in an *in vitro* system ($P>0.05$). Whereas, different processing temperatures affected on total gas, production of methane and NH_3-N concentration ($P\leq0.05$), increasing processing temperature decreased methane production. There were no interactions between biomass resources and processing temperature on *in vitro* rumen fermentation and methane production. These results implicate that rice husk and peanut shell derived biochar produced at 700ºC can be used to mitigate methane emission from cattle production, further *in vivo* studies are required to confirm practical parameters.

Keyword: Rice husk, Peanut shell, Biochar, *In vitro* fermentation, Methane production

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INTRODUCTION

Methane produced from cattle production by enteric fermentation process up to 85-90% and by excretion of feces (Ribeiro *et al.,* 2015). Bell *et al*. (2011) reported that 15% of global warming is due to enteric methane production. In addition, Johnson and Johnson (1995) concluded that, methane production is responsible for a loss of 2 to 12% of gross energy of dietary for the ruminants, which could be available used for growth or other performance. Therefore, reduction of methane emission from ruminants could bring about both environmental and economic benefits, with improving sustainable and energy efficient ruminant production, and in addition offer a potential way for global warming slowing (Cabeza *et al*., 2018).

Biochar is a byproduct produced from the pyrolysis of cellulose-rich biomass (Lehmann and Joseph, 2009), many previous studies reported that, the use of the biochar has the potential way to reduce methane production in cattle (Leng *et al*., 2012; Hansen *et al*., 2012; Saleem *et al*., 2018; Zhang *et al*., 2019). However, the mode of action of biochar is not fully understood (Winders *et al*., 2019). The reason is that the previous studies provided little or no detailed characteristics of the biochar (Cabeza *et al*., 2018). Some authors suggested that the gas adsorption by biochar in the rumen resulted in decreased methane belching, or the porous characteristic nature of biochar increases inert surface area in the rumen acknowledge for improved habitat of microbial, or change the community of microbial (Saleem *et al*., 2018; Leng, 2014). Furthermore, according to Sonoki *et al*. (2013) and Feng *et al*. (2012) when biochar is applied to the soil, the balance between methanotrophic and methanogenic organisms group was changed favorably toward decreasing the methanogen to methanotrophs ratio in paddy soil, and in the rumen this process may also occur (Winders *et al*., 2019). These functions of biochar are dependent on the characteristics of biochar. However, the characteristics of biochar are dependent on both the pyrolysis temperature and the sources of biomass from which it was produced (Tomczyk *et al*., 2020).

Vietnam is a tropical country with abundant crop byproducts, in which rice husk and peanut shell are dominant and there are potential biomass resources for biochar production. An estimated annual production of rice husk and peanut shell are 8.6 and 0.12 million ton, respectively (GSO, 2019). These byproducts were mainly used as fuel for cooking or burning on the field. This creates environmental pollution. However, so far, there have been few studies on processing and using biochar for ruminant production in Vietnam and in Asia as well. This study was conducted to determine effects of biochar produced from available tropical biomass resources of rice husk and peanut shell, and prepared at different processing temperatures on rumen fermentation characteristics and methane emissions *in vitro.*

MATERIALS AND METHODS

Materials: The experiment was carried out in the Lab Center of Animal Sciences and Veterinary Medicine Faculty, University of Agriculture and Forestry, Hue University, Hue city, Thua Thien Hue province, Vietnam. Two tropical biomass resources were used for producing biochar including rice husk and peanut shell. Biochar was produced at three processing temperatures of 300, 500 and 700^0C. Biochar was produced as described by Nguyen *et al.* (2018).

Experimental design: A 2 (biomass resources) x 3 (processing temperature) factorial design was used to study effects of biochar produced from rice husk and peanut shell, and prepared at different processing temperatures on characteristics of rumen fermentation and methane production *in vitro*. Biochar biomass resources included rice husk and peanut shell and biochar processing temperature included 300, 500 and 700^0C. Gas and methane production, dry matter (DM) and organic matter (OM) digestibility, and rumen fermentation characteristics (NH_3-N concentration and pH) were measured *in vitro*. These parameters were measured at three time points after the incubation (4, 24 and 48h). Total 90 bottles (2 biochar resources x 3 processing temperatures x 5 bottles/treatment combination x 3 time points) and 5 bottles for 5 bank samples were used for incubation.

Rumen inoculum: Ruminal fluid was collected from 4 fistulated beef cattle before the morning feeding in the farm of the Animal Sciences and Veterinary Medicine Faculty of Hue University of Agriculture and Forestry, Hue University. Cattle were fed diets consisting of forage (50%) and concentrate (50%). After being collected, the rumen fluids of 4 cattle were mixed and putted in a warmed thermos flask, then were quickly transferred to the lab where rumen fluid filtered through 4 layers of cheese and then carefully used buffer mineral solution to mix with rumen fluid with a ratio of of 1 part of remen fluid and 4 parts of buffer solution. All processes were made with anaerobic condition by flushing with CO_2 gas. Buffer solution as described by Theodorou *et al*. (1994) and was put in a water bath at 39^0C and used CO_2 gas to purged continuously for 30 minutes.

Substrates and chemical analyses: The feed substrate consisted of forage (50%) and concentrate (50%). Biochar was added to the feed substrate at 3% diet in DM basic. Using a hammer mill (Pullerisette 19, Fritsch GmbH, Laborgeratebau, Germany) to ground the substrates samples to pass a 1 mm sieve and then substrates was analysed chemical composition (DM, OM, CP, NDF).

***In vitro* fermentation and fermentation attributes analyses:** 250 mg of (the air-dried basis) substrate were incubated in the bottle (120 mL) which contained 25 mL of mixed rumen fluid and buffer mineral solution. The total gas production was determined at 4, 24 and 48h after incubation by using a manual pressure transducer combined with a syringe. Methane production was determined at the same time by Gas chromatography (Model 8610C Gas Chromatograph, SRI instruments Europe GmbH, USA).

Digestibilities of DM and OM, pH and concentration of NH_3-N were measured at 4, 24, and 48h after incubation. At each time point, pH value was determined immediately by pH meter (Hana, Germany), and then collection approximately 10 mL of end liquids and mixed with 0.2M HCl for analyses NH_3 -N concentration later. Centrifuge the rest of end liquids in each bottle at 10,000xg for 5 min, remove supernatant and dried at 105^0C for 12 h and burned at 550^0C for 4 h to measure DM and ash. DM digestibility and OM digestibility were determined as difference weight between after and before incubation, accurated by a blank which consisted of five bottles containing only rumen fluid and buffer solution. NH_3-N concentration was measured by method of AOAC (1990).

Statistical analysis: The effects of biomass resources and processing temperatures on gas and methane productions, *in vitro* digestibility of DM, OM, and *in vitro* rumen fermentation characteristics (pH and NH_3-N concentration) were analyzed using ANOVA of SPSS 16.0 with a statistical model following:

$Y_{ijk} = \mu + B_i + T_j + B_i*T_j + e_{ijk}$

Where Y_{ij} is the independent factor; μ is the overall mean; B_i is the effect of biomass resources; T_j is the effect of processing temperature; B_i*T_j is interaction between biomass resources and processing temperature; e_{ij} is the residual effect. Tukey test was used for pairwise comparison between two treatments when the P value of F test ≤0.05. In all the analyses, statistically significant differences were declared at P≤0.05.

RESULTS

Chemical composition of biochar and the substrates: The chemical composition of biochar is presented in Table 1. Biochar produced at higher temperature had a larger surface area and higher water holding capacity. When processing temperature increased from 300 to 500 and 700 ^{o}C, biochar surface area increased from 2.0, to 3.9 and 103.2 and from 2.3 to 3.1 and 101.0 m^2/g, respectively for rice husk and peanut shell; water holding capacity was increased from 3.1 to 3.6 and 5.2 and from 3.9 to 4.1 and 4.8, respectively. Rice husk derived biochar produced at different temperatures had higher ash concentration than peanut shell, from 20.7 to 24.7% compared to from 4.3 to 5.2%, respectively. In contrary, peanut shell derived biochar produced at different temperatures had higher organic matter concentration than rice husk, from 94.8 to 95.7% compared to 75.3 t0 79.3%, respectively (Table 1). The chemical composition of substrates when supplemented with 3% biochar is presented in Table 2. It can be seen from the table that substrates supplemented with 3% biochar had similar chemical compositions of DM, ash, OM, CP, EE and NDF.

Table 1. Chemical composition of biochar produced from tropical rice husk and peanut shell at different processing temparatures.

Biomass resources	**Rice husk**			**Peanut shell**		
Temperature (^{0}C)	**300**	**500**	**700**	**300**	**500**	**700**
DM (%)	98.2	92.2	97.4	98.8	97.2	97.4
Ash (%)	20.7	24.7	24.6	5.2	4.3	4.3
OM (%)	79.3	75.3	75.4	94.8	95.7	95.7
WC	3.1	3.6	5.2	3.9	4.1	4.8
C (%)	61.0	68.1	69.7	64.0	62.2	57.9
H (%)	1.8	1.7	2.3	3.3	4.4	1.7
O (%)	8.8	9.1	3.0	9.8	11.5	9.0
N (%)	1.1	0.4	0.4	1.1	0.6	0.4
P_2O_5 (%)	1.1	0.8	1.0	0.7	0.8	0.5
K_2O (%)	0.5	0.7	0.6	0.5	0.8	0.5
Surface area (m^2/g)	2.0	3.9	103.2	2.3	3.1	101.0
pH	9.14	9.22	8.92	9.21	9.51	9.01

DM: Dry matter; **OM:** Organic matter; **WC:** Water capacity

Table 2. Chemical composition of substrates when supplemented with 3% biochar.

Items	**Rice husk (RH)**			**Peanut shell (PS)**		
	RH300	**RH500**	**RH700**	**PS300**	**PS500**	**PS700**
DM (%)	88.8	89.2	89.3	88.9	89.4	89.6
Ash (%)	8.94	8.89	8.65	8.67	8.38	9.58
OM (%)	91.1	91.1	91.4	91.3	91.6	90.4
CP (%)	11.7	11.8	12.2	12.2	12.0	12.0
EE (%)	4.56	4.12	3.47	4.34	4.09	3.60
NDF (%)	50.7	47.4	47.1	52.6	52.3	50.2

DM: Dry matter; **OM**: Organic matter; **CP:** Crude protein; **EE:** Ether extracts; **NDF:** Neutral detergent fibre

Total gas and methane production: The total gas and methane production at 4, 24 and 48h after incubation are presented in Table 3. The biochar produced from rice husk, peanut shell had no significant effects on total gas and methane emission (P>0.05). Whereas, different processing temperatures had significant effects on total gas and methane emission (P≤0.05), increasing processing temperature decreased methane production. There were no interactions between biomass resources and processing temperature on total gas and methane emission (P>0.05).

Digestibility of DM and OM, pH value and NH_3-N concentration: The biochar produced from rice husk and peanut shell had no effects on *in vitro* digestibility of DM and OM, pH and concentration of NH_3-N (P>0.05). Whereas, different processing temperatures had a significant effect on concentration of NH_3-N (P≤0.05). Similar to total gas or methane emission, there were no interactions between biomass resources and processing temperature on *in vitro* digestibility, pH value and NH_3-N concentration at 4, 24 and 48h after incubation (Table 4).

Table 3. In vitro gas and methane production at 4, 24 and 48h after incubation.

Items	Biomass resources		Processing temperature			RSD	P-value		
	RH	PS	300	500	700		B	T	BxT
Gas production (ml/gDM)									
4h	33.1	34.3	35.1^{a}	34.4^{a}	31.6^{b}	1.141	0.055	0.008	0.226
24h	148.2	151.1	154.6^{a}	152.1^{a}	146.3^{b}	2.345	0.159	0.022	0.576
48h	229.3	230.3	234.0^{a}	231.0^{a}	224.4^{b}	7.210	0.791	0.004	0.269
CH_4 production (ml/gDM)									
4h	4.40	4.37	4.48^{a}	4.42^{a}	4.25^{b}	0.160	0.096	0.041	0.784
24h	23.7	23.5	24.8^{a}	23.4^{ab}	22.3^{b}	1.538	0.904	0.039	0.357
48h	34.1	35.4	36.8^{a}	34.9^{b}	32.5^{b}	1.722	0.801	0.002	0.921
CH_4/gas ratio									
4h	0.133	0.127	0.128	0.128	0.134	0.032	0.208	0.261	0.559
24h	0.160	0.156	0.160	0.155	0.152	0.045	0.179	0.406	0.619
48h	0.149	0.154	0.157	0.151	0.145	0.032	0.163	0.421	0.589

RH: Rice husk; **PS:** Peanut shell; **B:** Biomass; **T:** Temperature; **BxT:** Interaction between biomass resource and processing temperature; **RSD:** Residual standard deviation; ^{a,b} Means within rows within each factor missing a common superscript letter are different at $P \leq 0.05$.

Table 4. In vitro digestibility, pH and $N\text{-}NH_3$ concentration.

Items	Biomass Resources		Processing temperature			RSD	P-value		
	RH	PS	300	500	700		B	T	BxT
DM digestibility (%)									
4h	18.6	19.1	19.1	19.1	18.4	2.040	0.607	0.814	0.769
24h	50.4	50.9	50.0	50.4	51.7	2.197	0.600	0.413	0.821
48h	54.8	56.9	54.8	56.7	56.1	2.108	0.055	0.115	0.813
OM digestibility (%)									
4h	22.5	21.3	21.9	22.2	21.5	0.791	0.206	0.324	0.124
24h	55.2	54.3	53.9	55.4	55.1	1.533	0.221	0.234	0.180
48h	59.5	58.5	58.0	60.0	59.1	2.382	0.350	0.377	0.210
pH									
4h	6.92	6.87	6.85	6.86	6.87	0.044	0.120	0.810	0.224
24h	6.73	6.74	6.74	6.73	6.73	0.003	0.280	0.383	0.383
48h	6.66	6.68	6.73	6.67	6.61	0.032	0.129	0.872	0.328
NH_3-N concentration (mg/100mL)									
4h	5.23	5.21	5.16^{a}	5.22^{b}	5.30^{b}	0.084	0.660	0.041	0.471
24h	8.35	8.28	7.14^{a}	8.29^{b}	8.52^{c}	0.054	0.221	≤0.01	0.291
48h	8.43	8.43	8.27^{a}	8.44^{b}	8.58^{c}	0.071	0.350	≤0.01	0.359

RH: Rice husk; **PS:** Peanut shell; **B:** Biomass; **T:** Temperature; **BxT:** Interaction between biomass resource and processing temperature; **RSD:** Residual standard deviation; ^{a,b,c} Means within rows within each factor missing a common superscript letter are different at $P \leq 0.05$.

DISCUSSION

Effects of biochar on methane and total gas production in an *in vitro* system: Both rice husk and peanut shell are available agro-byproducts in tropical countries such as Vietnam. However, currently very few rice husk or peanut shell are used for any beneficial purposes, they are mainly burned and this process causes environmental pollution. Methane production from livestock should be mitigated to ensure sustainable development. Biochar has been declared for their potential of methane production reduction both in vivo and vitro studies (Leng *et al.*, 2012; Winders *et al.*, 2019). Therefore, using rice husk or peanut shell to produce the biochar should be considered. Biochar is not only used for reducing methane emission in livestock production (Cabeza *et al.*, 2018) but also as a potential strategy to mitigate of greenhouse gas when applied to soils (Gurwick *et al.*, 2013).

The present results indicate that the biochar produced from different tropical available biomass types of rice husk and peanut shell had no significant effects on methane and total gas production. This confirms findings of Cabeza *et al*. (2018); Hansen *et al*. (2012); McFarlane *et al*. (2017). Effects of biochar on gas and methane production depend on their physical and chemical characteristics (Hansen *et al*., 2012). The similar characteristics of biochar produced from rice husk and peanut huks (Table 1) could be the reason for this observation.

In this study, the effects of biochar produced from different processing temperatures on total gas or methane production were found. Cabeza *et al*. (2018) found that methane production decrease was higher with biochar produced at 550^0C than 700^0C. While, Calvelo Pereira *et al*. (2014) documented that there is no difference in methane production reduction between biochar produced at 350^0C or 550^0C. In the present study, methane production was decreased when biochar was produced at higher temperature. However, in general, no differences ($P>0.05$) were found between biochar prepared at 500^0C and 700^0C. The results of Cabeza *et al*. (2018), Calvelo Pereira *et al*. (2014) and this study showed that effects of biochar processed at different pyrolysis temperatures on methane production reduction were not consistent among studies. In current study, higher reduced methane production in biochar prepared at 500^0C and 700^0C compared with biochar prepared at 300^0C could be explained by different surface areas of biochar (Table 1), especially biochar prepared at 700^0C. High surface areas of biochar could help biochar adsorbed gases and/or methane emission (Hansen *et al*., 2012).

Inclusion of biochar has been shown to reduce methane production in cattle in both *in vitro* and *in vivo* condition (Leng *et al*., 2012; Hansen *et al*., 2012; Calvelo Pereira *et al*., 2014; Saleem *et al*., 2018; Cabeza *et al*., 2018; Winders *et al*., 2019). However, the mechanisms of their effects on methane production have not been explored clearly enough, although biochar has been shown as a potential methane mitigation strategy (Winders *et al*., 2019). The biochar used in these researches were prepared from different resources of biomass and at different processing temperatures, however their characteristics such as pH, surface area were not indicated (Calvelo Pereira *et al*., 2014; Leng *et al*., 2012; Hansen *et al*., 2012). Saquing *et al*. (2016) recommended that the acts of biochar as a redox-active mediator of electron that takes up electrons from microbial oxidation reaction and supply the electron at a certain distance from the center of microbial reaction, this process could reduce hydrogen in rumen. Previous studies recommended that biochar was prepared from rice husk at high temperatures with a high conductivity of electrical and capacity of electron buffering of fodder decomposing redox reactions (Yu *et al*., 2015; Sun *et al*., 2017). Sun *et al*. (2017) reported that different biochar has differences in conductivity of electrical and in capacity of electron buffering which depends on the biomass sources and pyrolysis temperature. Based on the above literature, the mitigation in methane was higher with biochar produced at 700^0C, or 500^0C than 300^0C in present study could be explained. First, higher surface area of biochar could adsorb more methane production. Second, biochar produced from high pyrolysis temperature has high electrical conductivity and electron buffering capacity of fodder decomposing redox reactions (Sun *et al*., 2017). Furthermore, according to Feng *et al*. (2012) and Sonoki *et al*. (2013) when biochar application in the soil, the balance between methanotrophic and methanogenic organisms group was changed unfavourably toward methanogenesis rather than methanotrophism. However, there is no evidence to prove the effectiveness of this process by different biochar types.

Effects of biochar on DM, OM digestibility, pH and NH_3-N concentration in an *in vitro* system: The biochar produced from different tropical available biomass resources had no different effects on *in vitro* DM and OM digestibility, pH and NH_3-N concentration. Similarly, biochar prepared at different pyrolysis temperature had no significant effects on *in vitro* DM and OM digestibility and pH value, however, had a significant effect on NH_3-N concentration. There were no interactions between biomass resources and processing temperature on *in vitro* DM, OM digestibility, pH and NH_3-N concentration. These results are similar with McFarlane *et al*. (2017) who reported that, *in vitro* digestibility was not differred by biochar resources. The pH value is an important parameter to monitor rumen fermentation (Kumar *et al*., 2013; Zhang *et al*., 2019). The pH value in this study is no differences among biochar resources, and ranged from 6.61 to 6.92, these pH values were higher than the 5.0 to 5.5 concluded by Hoover (1986) which activity of microbial in rumen was negatively affected. Zhang *et al*. (2019) documented that biochar supplementation leads to increased pH value due to the alkaline nature of the biochar. This means that the buffering capacity of biochar improved the suitable range for microbial activity. The effects of different biochar resources on NH_3-N concentration were not consistent in literature. Cabeza *et al*. (2018) reported that both processing temperature and biomass resource affected NH_3-N concentration. NH_3-N concentration were higher for biochar produced at 700^0C than 550^0C with biomass sources of rice husk, oil seed rape straw, *Miscanthus* straw and wheat straw. However, the concentration was lower for biochar prepared at 700^0C than 550^0C for the biomass of soft wood. They are in contrast to our present study in which concentrations of NH_3-N were higher for biochar prepared at 700^0C than 550^0C or 300^0C, that is in agreement with the findings of Cabeza *et al*. (2018). The effect of biochar on concentration of NH_3-N could be explained that biochar adsorb NH_3-N production (Cabeza *et al*., 2018), while Gai *et al*. (2014) and Winnin (2014) reported that the efficacy of adsorbing NH_3-N by biochar was no improved by the pyrolysis temperature, increasing processing temperature decrease cation exchange capacity. This may be the reason why NH_3-N concentration is higher for biochar produced at 700^0C than 550^0C or 300^0C in the present study. The NH_3-N concentrations in this experiment were higher 5 mg/100 mL, which is required to improve the growth rate of bacteria in rumen for the optimal feed fermentation and digestion of DM and OM in the rumen (McDonald *et al*., 1995).

Conclusions: The biochar produced from rice husk and peanut shell had no significant effects on gas, methane production, *in vitro* digestibility of DM and OM, pH and concentration of NH_3-N. Whereas total gas, methane production and NH_3-N concentration were significantly affected by different processing temperatures, increasing processing temperature decreased methane production and concentration of NH_3-N. There were no interactions between biomass resources and processing temperature on total gas and methane production. These results highlight the advantages and need for more research in animals (*in vivo* condition) to understand and confirm the practical parameters.

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