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ORIGINAL ARTICLE

Effects of levels of tropical rice husk-derived biochar in diet-based high rice straw on in vitro methane production and rumen fermentation

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Abstract
This study was conducted to evaluate effects of biochar levels in diets on in vitro methane emission and rumen fermentation. Biochar, produced from rice husk, pyrolysis at 700 °C, was supplemented in diets at 0, 1, 3, 5, and 7% (dry matter (DM) basis). Total gas, methane production, dry matter (DM) and organic matter (OM) digestibility, pH, and NH_3-N concentration were measured at 4, 24, and 48 h after incubation. Results showed that total gas and methane emission of diets with biochar was lower than that of the diet without biochar. Methane production decreased linearly ($P<0.01$) with increasing biochar levels. DM and OM digestibility, as well as NH_3-N concentration of diets with 1 or 3% biochar, was similar to the diet without biochar. When the dietary biochar level was increased up to 5 or 7% in diets, OM and DM digestibility and NH_3-N concentration decreased significantly compared to the diet without biochar ($P<0.01$). Our findings implicated that supplementing 3% biochar (DM basis) to the diet is recommended to reduce methane production.

Keywords Biochar · In vitro fermentation · Methane · Rice husk

1 Introduction

Methane, a radiatively active gas, is one of the greenhouse gases which causes climate change, it accounted for 10% of global greenhouse gas in 2016 [1]. Methane is naturally emitted to the atmosphere from various sources, such as wetlands, ocean floors, or swamps (as the anaerobic bacterial decomposition of vegetable matter underwater) [2]. However, it is also produced as a result of anthropogenic activities including slurries and enteric fermentation in livestock production [3, 4]. Ribeiro et al. [5] reported that 85–90% of the methane produced from cattle is from enteric fermentation, and enteric methane is responsible for 15% of global warming [6]. In ruminants, methane emission also causes a loss of gross energy of the diet [7]. Therefore, increasing ruminant performance and reducing methane production is an important strategy, which aims to use energy efficiently for animals and to reduce the effect of climate change [8].

According to Yaashikaa et al. [9], biochar is a product made by pyrolysis of lignocellulosic biomass that includes agricultural and forestry residues, agro-industrial wastes, short rotation forestry, and dedicated energy crop, which constitutes a mixture of natural polymers, namely cellulose, hemicellulose, and lignin. Previous studies showed that supplementing biochar in the diet could decrease methane production under in vitro as well as in vivo experiments, and improve animals' production, growth, immunity, blood profile, and thus the overall agricultural productivity [8, 10–13]. Different biomass sources (e.g., rice straw, rice husk, corncob, peanut shell) were

Highlights
- Methane production decreased linearly with increasing biochar levels;.
- DM and OM digestibility, as well as NH_3-N concentration of diets with 1 or 3% biochar, were similar to the diet without biochar;.
- When dietary biochar level was increased up to 5 or 7% in diets, OM and DM digestibility and NH_3-N concentration was decreased;.
- Supplementing 3% biochar (DM basis) to the diet is recommended to reduce methane production.

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used to produce biochar and supplemented to diets to reduce methane production [14, 15]. The balance between the group of methane-producing microorganisms (methanogenic) and the group of methane-using microorganisms (methanotrophic) was changed when supplementation of biochar favorably toward methanotrophic rather than methanogenesis, higher the methanotrophic group will increase the methane oxidation process, thereby reducing the methane accumulation [16, 17]. In addition, according to Sun et al. [18], high-temperature pyrolysis biochar has a high conductivity of electricity and the capacity of electron buffering of fodder decomposing redox reaction. Furthermore, the most important reason which biochar can decrease methane production is its ability to absorb and adsorb gasses [19]. Biochar has a high surface area; this could help biochar adsorb and absorb gases and/or methane production [20].

The type of biomass used significantly affect the porosity, surface area, and internal structure of biochar, all of which influence its immobilization and sorption capabilities [21]. Lignocellulosic materials are mainly composed of varying levels of cellulose, hemicellulose, lignin, and inorganic matter. Pyrolysis of each of the main constituents follows unique reaction pathways that highly influence the yield and characteristics of the resulting biochar. Biomass with high lignin content usually exhibits high biochar yield, high carbon content, high specific area, and a more aromatic carbon structure [22]. Rice (*Oryza sativa* L.) is a major cereal crop used for human nutrition. Harvesting and processing of rice generate huge amounts of lignocellulosic byproducts such as rice husks and straw, which present important lignin contents that can be used to produce biochar. Rice husk has higher than rice straw [23, 24]. Therefore, rice husk can be a potential feedstock for biochar production.

From the literature, it could be confirmed that dietary biochar supplementation can reduce methane production compared to without supplementation. However, effects of supplementing different biochar levels in general and rice husk-derived biochar in particular to diets on methane production were not much studied. This present study was aimed at evaluating effects of levels of tropical rice husk-derived biochar in diets on in vitro methane production and rumen fermentation characteristics.

2 Methods

2.1 Materials

The experiment was conducted at the Lab of the Faculty of Animal Sciences and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue city, Thua Thien Hue province, Vietnam.

Biochar was produced from tropical rice husk and pyrolysis at 700 °C according to the procedure described by Nguyen et al. [25]. The biochar characteristics were 97.4% dry matter (DM), 75.3% organic matter (OM), 69.7% C, 2.3% H, 3.0% O, 0.4% N, 1.0% P_2O_5, and 0.6% K_2O; the surface area was 103.2 m^2/g, water holding capacity was 5.2 and pH was 8.92.

2.2 Experimental design

A complete randomized design was used to determine effects of rice husk biochar levels in diets on in vitro methane emission and rumen fermentation characteristics. There were five treatments of five levels of biochar in diets: 0, 1, 3, 5, and 7% (DM basis), one treatment with five replications. In vitro total gas and methane productions, DM and OM digestibility, and rumen fermentation characteristics (pH and NH_3-N concentration) were measured at 4, 24, and 48 h after incubation. A total of 80 bottles (5 levels of biochar × 5 bottles/treatment combination × 3-time points and 5 bottles for 5 blank samples) were used for incubation.

2.3 Rumen inoculum

Four 4 fistulated beef cattle were fed diets consisting of concentrate (30%) and forage (70%) for 14 days before rumen fluid collection. Rumen fluid was collected before the morning feeding. It was then transferred to the laboratory. In the laboratory, the rumen fluid of 4 cattle was mixed and placed in the incubator (39 ± 0.5 °C). After that, the rumen fluid was filtered through 4 layers of screen cloth to remove feed particles and mixed with a buffer solution at a ratio of 1:4. The buffer mineral solution was used as recommended by Theodorou et al. [26]. All stages of sample processing were carried out under anaerobic conditions through aeration of CO_2.

2.4 Substrates and chemical analyses

The feed substrate used in in vitro fermentation consisted of rice straw (70%) and concentrate (30%). Biochar was supplemented in the feed substrate at 0, 1, 3, 5, and 7% as DM basic. Feed samples were crushed at 1 mm by using a hammer mill. The ingredients and chemical composition of the substrate was analyzed and presented in Table 1.

2.5 In vitro fermentation and data collection

Two hundred fifty milligrams of the substrate (air-dried) was added into a 120-mL bottle contained 25 mL of mixed buffer mineral solution and rumen fluid. During the incubation, the gas production was measured at 4, 24, and 48 h using a syringe combined with a pressure transducer (Kimo, France). Methane concentration was also measured at 4, 24,

Table 1 Ingredients and chemical composition of substrates

Items	Biochar levels in diet (%DM)				
	0	1	3	5	7
Ingredients					
Rice straw	70	70	70	70	70
Soybean meal	15	15	15	15	15
Maize powder	8	8	7	6	5
Rice bran	7	6	5	4	3
Biochar	0	1	3	5	7
Total	100	100	100	100	100
Chemical composition (%DM)					
DM	87.9	88.0	88.1	88.2	88.2
Ash	10.3	10.5	10.7	11.0	11.1
OM	89.7	89.5	89.3	89.0	88.9
CP	12.0	11.9	11.7	11.8	11.6
EE	5.70	5.57	5.59	5.41	5.32
NDF	50.3	50.1	49.9	49.6	49.7
ADF	32.0	31.9	31.8	31.6	31.5

CP, crude protein; *DM*: dry matter; *EE*, ether extracts; NDF, neutral detergent fiber; *OM*, organic matter

and 48 h by using gas chromatography (Model 8610 gas chromatograph, SRI intruments Europe GmbH, USA).

In vitro DM and OM digestibility, pH, and NH_3-N concentration were determined at 4, 24, and 48 h after incubation. At each time, we open the cap of the bottle and determine the pH value immediately with a handheld pH meter (Hana, Germany), and then, about 10 mL of the final liquids was sampled and divided into aliquots for analyses NH_3-N concentration after being well mixed with 0.2 M HCl. The remainder in each bottle was centrifuged at 10,000 × g for 5 min, then removed all the supernatant; the remaining residue was dried at 105 °C for 12 h to determine DM. After weighing to determine DM, continue burning at 550 °C for 4 h to determine Ash. The digestibility of DM and OM was calculated based on the difference between the weight before and after incubation. NH_3-N concentration was determined by the AOAC method [27].

2.6 Statistical analysis

Effects of biochar levels on in vitro total gas and methane production, DM and OM digestibility, pH, and NH_3-N concentration were analyzed using SPSS 16.0 software with the following model.

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is each observation from biochar level i, bottle j; μ is the overall mean; T_j is the biochar level effect; e_{ij} is error term. The Tukey test was used to compare the difference between each pair of treatment. Significant effects were declared at $P < 0.05$.

3 Results

3.1 Total gas and methane production

Total gas and methane production after 4-, 24-, and 48-h incubation were significantly affected by different biochar levels ($P < 0.01$). Increased biochar levels linearly reduced total gas and methane production (Table 2). Total gas and methane in the diet with biochar were lower than that in the diet without biochar.

3.2 In vitro DM, OM digestibility, pH, and NH_3-N concentration

DM and OM digestibility was significantly affected by increasing the biochar level in diets ($P < 0.05$). pH did not vary significantly among different biochar levels in the diet ($P > 0.05$), whereas NH_3-N concentration differed among treatments and decreased linearly ($P < 0.05$) when dietary biochar levels were increased (Table 3). DM and OM digestibility, as well as NH_3-N concentration of diets supplemented with 1 or 3% biochar, was similar to the diet without biochar. When dietary biochar supplementation was increased to 5 or 7%, DM and OM digestibility, N-NH_3 concentration, decreased significantly compared to the diet without biochar.

4 Discussion

The supplementation of biochar to the diet had a potential to reduce methane production as reported by previous studies [8, 11, 12, 20, 28]. However, effects of dietary biochar levels in general and tropical rice husk-derived biochar in particular on methane production, nutrient digestibility, and rumen fermentation characteristics have not much studied. Therefore, it is essential to study effects of different tropical rice husk-derived biochar levels supplemented to diets on methane production and rumen fermentation characteristics. Our study showed that after 4, 24, and 48 h of incubation average methane production decreased by 9.8, 14.8, 16.9, and 17.03%, respectively for 1, 3, 5, and 7% biochar diets compared to the diet without biochar. This reduction is in the range of the findings of previous studies. Leng et al. [29] concluded that in vitro methane production was decreased by 12% when adding 1% of biochar to the diet. Phanthavong et al. [30] reported that the diet supplemented biochar (1% DM basis) reduced in vitro methane production after 24-h

Table 2 Effects of biochar levels in diet on total gas and methane production at 4, 24, and 48 h after the incubation

Items	Biochar levels in diet (%DM)					SEM	Contrast[1]		
	0	1	3	5	7		T	L	Q
Gas production (mL/g DM)									
4 h	30.0^{a}	29.8^{ab}	29.0^{b}	28.2^{c}	27.8^{c}	0.282	<0.001	<0.001	0.709
24 h	148.2^{a}	142.6^{a}	131.6^{b}	128.7^{b}	129.6^{b}	1.976	<0.001	<0.001	0.010
48 h	220.0^{a}	210.8^{b}	206.9^{bc}	204.0^{cd}	199.5^{d}	2.040	<0.001	0.002	0.188
CH_4 production (mL/g DM)									
4 h	4.61^{a}	4.19^{ab}	3.92^{b}	3.92^{b}	3.98^{b}	0.143	0.012	0.003	0.030
% reduction[2]	-	9.11	14.97	14.97	13.67	-	-	-	-
24 h	25.1^{a}	22.4^{b}	20.3^{c}	19.2^{d}	19.6^{cd}	0.367	<0.001	<0.001	<0.001
% reduction	-	10.76	19.12	23.51	21.91	-	-	-	-
48 h	33.5^{a}	30.3^{b}	30.0^{bc}	29.4^{bc}	28.3^{c}	0.616	<0.001	<0.001	0.106
% reduction	-	9.55	10.45	12.24	15.52	-	-	-	-

DM, dry matter; [1]treatment (T), linear (L), and quadratic (Q) effects of different biochar levels; *SEM*, standard error of the mean, [2]compared to the treatment without biochar, [a–d]within each row, the numbers with different superscript letters are statistically different $P < 0.05$

incubation by 6.5% compared to the diet without biochar. Hansen et al. [20] reported that in vitro methane production was decreased between 11 and 17% when adding 9% w/w biochar to the diet.

Our studies also confirmed previous in vivo studies. Leng et al. [31] concluded that methane production reduced between 11 to 13% when supplementing 1.0% biochar to cattle diets. Cattle fed the diet with 1.0% biochar produced less methane than the diet with 0.5 biochar from 1 to 2.3% [10]. Winders et al. [12] found that cattle fed the diet with 3.8% biochar decreased methane emission between 9.9 to 18.4% compared to the diet without biochar.

Methane production reduction as a result of biochar supplementation to the diet can be explained. Feng et al. [16] and Sonoki et al. [17] reported that when supplementation of biochar, the balance between the group of methane-producing microorganisms (methanogenic) and the group of methane-using microorganisms (methanotrophic) was changed favorably toward methanotrophic rather than methanogenesis when biochar was supplemented. Man et al. [1] reported that methanogenic archaea and methanotrophic proteobacteria are the important bacteria responsible for methane emission, the methanotrophic group increased results in the methane oxidation increased, thereby methane accumulation was reduced. In addition, the supplementation of biochar provides habitat and stimulates the growth of methanotrophic bacteria [29]. Furthermore, Sun et al. [18] documented that high-temperature pyrolysis biochar has a high conductivity of electricity and capacity of electron buffering of fodder decomposing redox reactions. Danielsson et al. [19] recommended that the most important reason biochar decreases methane production is its ability to absorb and adsorb gasses. The characteristic of biochar is high surface area; therefore, biochar could adsorb methane and/or gas production [20]. In the present study, the surface area of biochar used was 103.2 m^2/g; this could be an important reason for the reduction of methane production.

In the context of climate change mitigation, there is an urgent need to capture and sequester carbon from the atmosphere. Production, use, and storage of biochar can have an estimated sequestration of 0.3–2 Gt CO_2 $year^{-1}$ by 2050 [32]. Biochar eligibility (yield, chemical properties, physical properties, hydrological properties, stability) is highly dependent on the type of feedstock utilized and processing conditions employed (temperature, residence time, particle size, carrier gas, heating rate, pressure, biochar engineering). Carbon removal services via biochar are currently offered through marketplaces that require certification. Certifications are prerequisites for the eligibility of biochar producers to become participants within the carbon removal marketplace. The European biochar certificate and International biochar initiative in the USA are the two well-recognized standards. The European biochar certificate is a

Table 3 Effects of biochar levels in diet on in vitro digestibility, pH, and NH_3-N concentration

Items	Biochar levels in diets (% DM)					SEM	Contrast[1]		
	0	1	3	5	7		T	L	Q
DM digestibility (%)									
4 h	19.0^{a}	19.1^{a}	18.7^{a}	17.1^{b}	16.5^{b}	0.412	< 0.001	< 0.001	0.097
24 h	50.9^{a}	50.5^{a}	50.0^{a}	47.3^{b}	45.0^{b}	0.850	< 0.001	< 0.001	0.071
48 h	58.7^{a}	59.1^{a}	58.8^{a}	54.1^{b}	54.8^{b}	0.677	< 0.001	< 0.001	0.163
OM digestibility (%)									
4 h	23.2	20.9	21.1	18.2	19.0	2.470	0.651	0.172	0.742
24 h	54.7^{a}	55.8^{a}	55.8^{a}	53.7^{a}	51.9^{b}	0.735	0.007	0.004	0.010
48 h	60.7^{a}	60.8^{a}	59.7^{a}	56.2^{b}	55.5^{b}	0.460	< 0.001	< 0.001	0.033
pH									
4 h	6.83	6.74	6.85	6.85	6.83	0.037	0.265	0.387	0.828
24 h	6.76	6.72	6.74	6.71	6.74	0.023	0.609	0.263	0.220
48 h	6.68	6.67	6.71	6.66	6.71	0.018	0.198	0.410	0.485
NH_3-N concentration (mg/100 mL)									
4 h	5.93^{a}	5.85^{a}	5.62^{ab}	5.20^{b}	5.11^{b}	0.186	0.015	0.001	0.768
24 h	9.73^{a}	9.75^{a}	9.31^{b}	8.80^{c}	8.56^{d}	0.051	< 0.001	< 0.001	0.005
48 h	10.2^{a}	10.1^{a}	9.98^{a}	8.90^{b}	8.71^{b}	0.263	0.001	< 0.001	0.252

DM, dry matter; [1]treatment (T), linear (L), and quadratic (Q) effects of different biochar levels; *SEM*, standard error of the mean, $^{a–d}$within each row, the numbers with different superscript letters are statistically different $P < 0.05$

well-recognized voluntary body setting standards for sustainable biochar production in Europe. The guidelines developed provide all necessary requirements and details for the certification of biochar producers. The certification process includes an overall assessment on feedstock eligibility, production process eligibility and requirements, sampling, labeling and quality management procedures, health and safety regulations, and most importantly biochar properties. Properties must meet regulatory thresholds. The international biochar initiative is a voluntary biochar certification and standard setting entity operating in the USA. Similar to the European biochar certificate, the international biochar initiative has developed standards and procedures for the certification of biochar producers. The standards cover feedstock eligibility, declaration of biochar properties and regulatory thresholds, general protocols and restrictions, and recommendations on best management practices for production, handling, and storage [33]. In our study, supplementing rice husk-derived biochar to the diet reduced methane production. However, to allow rice husk-derived biochar producers to become a participant within carbon removal market place, further research on feedstock eligibility, production process eligibility and requirements, and biochar properties that meet standards such as European biochar certificate or the international biochar initiative are necessary.

Our study found that the supplementation of biochar at 1 or 3% in the diet did not affect DM and OM digestibility compared to the diet without biochar; however, when increasing dietary biochar up to 5 or 7%, DM and OM tended to decrease. This confirmed the finding of Winders et al. [12] who reported that adding 0.8 or 3% biochar to the diet did not affect DM and OM digestibility. Our results were also consistent with the finding of McFarlance et al. [34] who reported that high-level biochar supplementation (8% DM) reduced DM digestibility. Mengistu et al. [35] also report that biochar levels in diet (2.25 or 4.5%) did not affect DM digestibility. However, our studies were not consistent with the findings of some studies of Van et al. [36]; Leng et al. [29]; and Saleem et al. [11] who reported that DM and OM digestibility was improved when diets were supplemented with biochar, whereas Hansen et al. [20] and Winders et al. [12] reported that biochar supplementation to the diet did not affect DM digestibility. Inconsistent effects of biochar supplementation on DM and OM digestibility might be due to the fact that different sources of biomass, pyrolysis conditions, and particle size were used among studies. These affect fermentation activities and thus digestibility [11].

The rumen pH is an important indicator of rumen health [37, 38]. Our study showed that the pH was similar among treatments with different biochar levels, ranging from 6.66 to 6.85. The pH in this study was higher than the pH range of 5.0–5.5 in which the ruminal microbial activity was affected [39]. Zhang et al. [38] reported that supplementation of biochar in the diet resulted in increased pH value due to the alkaline properties of biochar. This means, the suitable range for the microbial activity in the rumen was improved when biochar was supplemented to the diet. The concentration of NH_3-N in the present study was decreased linearly with increasing biochar levels. There is still a lack of information about the impact of biochar supplementation on NH_3-N concentration. However, the reduction of the concentration of NH_3-N in this study could be explained that NH_3-N was adsorbed by biochar as reported by Cabeza et al. [8], and increased biochar levels result in reduced DM and OM digestibility, thereby reducing NH_3-N in incubation. The concentration of NH_3-N in this study was higher than 5 mg/dL, which is necessary for microorganisms' growth in the rumen in order to optimize the fermentation process and improve the digestibility of OM in the rumen [40].

5 Conclusions

Methane production was decreased linearly as biochar supplementation to the diet was increased. DM and OM digestibility and NH_3-N concentration in diets with 1 or 3% biochar were similar to the diet without biochar. When the dietary biochar level was increased higher in the diets (5 or 7%), the OM and DM digestibility and NH_3-N concentration decreased significantly compared to the diet without biochar ($P < 0.01$). Our findings implicated that supplementing 3% biochar (DM basis) to the diet is recommended to reduce methane production, but not affect the OM and DM digestibility.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13399-022-03431-y.

Author contribution Dinh Van Dung: conceptualization, methodology, analysis, writing original, and revising manuscript. Le Dinh Phung: conceptualization, methodology, and revising manuscript. Hynek Roubík: conceptualization, and revising manuscript. Le Duc Ngoan: giving comments on the manuscript.

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Declarations

Ethical approval The experiment used four fistulated beef cattle for rumen fluid collection. The experimental procedures followed the Ethical guidelines of the Animal Ethics Committee of Hue University, Hue city, Vietnam (HUVN0009).

Competing interest The authors declare no competing interests.

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