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ORIGINAL ARTICLE

Effects of biochar produced from tropical rice straw, corncob, and bamboo tree at different processing temperatures on in vitro rumen fermentation and methane production

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Abstract

This study aimed to evaluate the effects of biochar produced from tropical biomass resources (rice straw, corncob, and bamboo) at different processing temperatures (300, 500, and 700 °C) on in vitro rumen fermentation and methane production. Treatments were arranged as a 3 × 3 factorial design with three biomass resources and three biochar processing temperatures. Added biochar occupied 3% of the substrate (DM basic). Two hundred fifty milligrams of the air-dried substrate was incubated in 120 ml bottles, which contained 25 ml of mixed rumen fluid and buffer mineral solution. Total gas and methane production, in vitro digestibility of DM and OM, and in vitro rumen fermentation characteristics were determined at three time points of 4, 24, and 48 h of the incubation. The results showed that biomass resources and processing temperatures affected gas production at 4, 24, and 48 h after incubation ($P < 0.01$). Interactions between biomass resources and processing temperatures affected gas production at 4 h ($P = 0.06$) and 24 h ($P = 0.001$). Biomass resources and processing temperatures affected methane production at different time points of the incubation ($P < 0.05$), except the effect of biomass resources at 24 h ($P = 0.406$). Increased processing temperature from 300 to 700 °C reduced gas and methane production ($P < 0.05$). Biomass resources affected OM digestibility after 4 and 24 h of incubation. Processing temperatures and their interaction with biomass resources affected OM digestibility after 48 h of incubation ($P < 0.001$). NH_3-N concentrations at 24 and 48 h were highest for corncob, then rice straw, and lowest for biochar derived from bamboo tree ($P < 0.05$). Increased processing temperatures resulted in higher NH_3-N concentrations at 24 and 48 h of incubation ($P < 0.05$). To mitigate methane production, biomass resources and processing temperatures should be considered when using biochar as a feed additive in ruminant diets.

Keywords Greenhouse gasses · Biomass · Environment · Biochar

Highlights
- Biomass resources and processing temperatures affect gas and methane production.
- Total gas and methane production is higher for corncob than rice straw and bamboo tree-derived biochars.
- Increasing processing temperature from 300 to 700 °C reduces gas and methane production.
- NH_3-N concentrations are highest for corncob, then rice straw, and lowest for bamboo tree–derived biochar.
- Increasing processing temperatures results in higher concentrations of NH_3-N.

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1 Introduction

Ruminant production accounts for about 81% of total greenhouse gas (GHG) from the livestock sector [1]. Gaseous excretions mainly produce ruminants' GHG emissions through eructation and exhalation. Methane emissions, mainly produced through rumen microbial methanogenesis, are responsible for 90% of the GHG caused by cattle [2]. Archaea carry methanogenesis that converts microbial fermentation products of H_2 and CO_2 or formate to methane. Hydrogen serves as an electron donor for the microbial reduction of CO_2 to methane. Methane production and emission mean a loss of energy for the animal, ranging from 2 to 12% of total gross energy intake [3]. The production of methane is implicated in global warming [4]. Thus, it should be reduced. Nitrate and sulfate, other electron acceptors in addition to CO_2 and enteric fatty

acids, react with ammonia and hydrogen sulfide, respectively. However, they are toxic for the animals at higher concentrations.

Biochar is the carbon-rich solid product of pyrolysis, which is the thermal decomposition of biomass at high temperatures with little or no oxygen present [5]. Biochar could act as an electron acceptor and thus reduce methane production in the rumen [6]. Supplementing biochar (with and without biochar, different levels of biochar) has shown to decrease from 5 to 25% methane production, both in vitro and in vivo experiments [7–10]. Different experiments used biochars produced from various biomass resources and at different processing temperatures. Different types of biochar among the experiments could be the reason for this high variation in the reduction in methane production. Biomass resources and pyrolysis temperature determine biochar's properties, thus methane production reduction [11]. Vietnam is rich in tropical biomass resources such as rice straw, corncob, and bamboo trees that can be used to produce biochar. This research was aimed at evaluating the effects of biochar produced from tropical rice straw, corncob, and bamboo tree at different processing temperatures on total gas, methane production, in vitro digestibility, and rumen characteristics.

1.1 Methods

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

1.1.1 Materials

The experiment was carried out at the Center for Lab Animal Sciences and Veterinary Medicine of the Faculty of Agriculture and Forestry (HUAF), Hue University, Hue city, Thua Thien Hue province, Vietnam. Three tropical biomass resources were used for producing biochars including rice straw, corncob, and dry bamboo tree. These are available materials in Vietnam; all of them were bought from farmers in the Central region, Vietnam, and stored at the Faculty of Animal Sciences and Veterinary Medicine, HUAF, before producing biochar. Each biochar type was produced at three processing temperatures (300, 500, and 700 °C). Biochars were produced as described by Nguyen et al. [12]. The chemical composition and characteristics of the biochars are presented in Table 1. Remarkably, biochars produced from different tropical biomasses have differed in surface areas (m^2/g), and increased processing temperatures resulted in increased surface areas of biochars.

Table 1 Chemical composition of biochar produced from tropical rice straw, corncob, and bamboo tree at different processing temperatures

Items	R			C				B		
	R300	R500	R700	C300	C500	C700	B300	B500	B700
DM, %	98.0	94.7	96.6	99.7	93.9	97.5	99.1	95.3	95.9
Ash, %	18.0	17.3	21.3	3.6	3.5	3.9	9.5	10.5	11.4
OM, %	82.0	82.7	78.7	96.4	96.5	96.1	90.5	89.5	88.6
WC, %	4.8	4.4	6.6	3.2	3.4	5.5	3.6	3.9	5.8
C, %	61.0	62.4	69.4	67.1	66.1	59.3	57.2	59.1	56.8
H, %	1.9	1.6	1.4	2.0	2.5	1.7	1.7	2.9	2.0
O, %	10.6	10.2	8.7	11.3	12.6	7.8	6.5	5.0	7.9
N, %	0.7	0.7	0.6	0.5	1.0	0.8	0.5	0.7	0.3
P_2O_5, %	1.0	0.7	0.6	0.8	1.0	0.6	0.6	1.0	1.0
K_2O, %	0.6	0.7	0.7	0.6	0.7	0.4	0.9	0.7	0.6
Surface area, m^2/g	2.7	15.7	211.6	1.1	7.1	98.6	5.4	63.0	154.2
pH	8.78	8.91	9.02	8.99	8.97	9.21	9.22	9.03	8.99

R, rice straw; *C*, corncob; *B*, bamboo; *DM*, dry matter; *OM*, organic matter; *WC*, water capacity

1.1.2 Experimental design

A 3 biomass resources × 3 processing temperature factorial design was used to study the effects of biochars produced from rice straw, corncob, and bamboo tree, produced at different processing temperatures on in vitro rumen fermentation characteristics and methane production. Biochar biomass resources included rice straw, corncob, and bamboo tree and biochar processing temperature included 300, 500, and 700 °C. Total gas and methane production, dry matter digestibility (DM), organic matter (OM), and fermentation characteristics of rumen in vitro (pH and concentration of NH_3-N) were determined at 3 time points (4, 24, and 48 h after incubation). Total 140 bottles (3 biochar resources × 3 processing temperatures × 5 bottles/treatment combination × 3 time points and 5 bottles for 5 blank samples) were used for incubation.

1.1.3 Rumen inoculum

Rumen fluid was collected before the morning feeding 4 fistulated beef cattle at the Faculty of Animal Sciences and Veterinary Medicine farm, Hue University of Agriculture and Forestry. Cattle were fed diets consisting of rice straw (50%) and concentrate (50%), concentrate comprising soybean meal (25%), maize meal (25%), rice bran (30%), and cassava powder (20%). After being collected, the rumen fluids were immediately transferred to the laboratory where the rumen fluid of 4 cattle was mixed and placed in a warmed thermos flask (39 ± 0.5 °C), and then filtered through 4 layers of cheesecloth to eliminate feed particles and then carefully mixed with the buffer mineral solution with a ratio of 1 part rumen fluid and 4 parts buffer solution. All operations were carried out under anaerobic conditions by flushing with carbon dioxide. Buffer mineral solution, as described by Theodorou et al. [13], was preheated in a water bath at 39 °C and purged continuously with CO_2 for 30 min.

1.1.4 Substrates and chemical analyses

The substrate ingredients are presented in Table 2. The added biochar occupies 3% of the substrate (DM basis). Substrate samples were ground to pass a 1-mm sieve using a hammer mill (Pullerisette 19, Fritsch GmbH, Laborgeratebau, Germany) and then analyzed for DM, ash, and ether extracts (EE) according to the standard methods of the Association of Official Chemists (AOAC) (1990). The neutral detergent fiber (NDF) was analyzed as described by Van Soest et al. [14]. The N was analyzed according to AOAC [15] and the CP concentration was calculated as N × 6.25. The chemical composition of the substrate is presented in Table 3.

Table 2 Substrate ingredients (% of DM basis)

Ingredients	%
Rice straw	50
Soybean meal	15
Maize meal	12
Rice bran	10
Cassava powder	10
Biochar	3

1.1.5 In vitro *fermentation and fermentation characteristic analyses*

Two hundred fifty milligrams of the air-dried substrate was incubated in 120 ml bottles, which contained 25 ml of mixed rumen fluid and buffer mineral solution. Total gas production was measured at 4, 24, and 48 h during incubation using a manual pressure transducer (Digitron 2023P, Digitron, Torquay, Devon, UK) combined with a syringe. Methane production was determined simultaneously by gas chromatography (Model 8610C gas chromatograph, SRI instruments Europe GmbH, USA).

Digestibility of DM and OM, pH, and NH_3-N concentration were determined at 3 time points (4, 24, and 48 h of incubation). Each time, the pH value was measured immediately with a pH meter (Model HI8314, Hana, Rumania). Approximately 10 ml of end liquids was sampled and divided into aliquots for downstream NH_3 -N concentration analyses after being equally mixed with 0.2 M HCl. The rest of the end liquids in each bottle were centrifuged at 10.000 × g for 5 min. The supernatant was removed and dried at 105 °C for 12 h and burned at 550 °C for 4 h to determine DM and ash concentration. Digestibility of DM and OM was calculated as the difference of weight before and after the incubation, corrected by blank samples, which consisted of five flasks containing only buffered rumen fluid. NH_3-N concentration was measured by the method of AOAC [15].

1.1.6 Statistical analyses

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

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

where Y_{ijk} is the observation from bottle j; μ is the overall mean; B_i is the effect of biomass resources; T_j is an effect of processing temperature; $B_i{*}T_j$ is an interaction between

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

Items	R			C				B	
	R300	R500	R700	C300	C500	C700	B300	B500	B700
DM, %	88.5	89.5	89.2	88.7	89.6	89.9	88.8	89.6	89.4
Ash, %	9.11	7.98	8.51	9.69	7.75	7.63	9.15	8.15	8.19
OM, %	90.9	92.0	91.5	90.3	92.2	92.4	90.8	91.8	91.8
CP, %	12.0	11.7	12.2	12.4	11.4	11.8	11.7	11.7	12.1
EE, %	3.90	3.74	3.30	4.50	4.26	3.68	4.50	4.37	3.72
NDF, %	57.2	51.5	46.7	52.9	50.1	52.0	50.1	50.7	54.2

R, rice straw; *C*, corncob; *B*, bamboo; *DM*, dry matter; *OM*, organic matter; *CP*, crude protein; *EE*, ether extracts; *NDF*, neutral detergent fiber

biomass resources and processing temperature; and e_{ijk} is the residual effect. The Tukey test was used for a pairwise comparison between two treatments when the *P*-value of the *F* test was < 0.05. In all the analyses, significant effects were declared at $P < 0.05$.

2 Results

2.1 Gas and methane production

The effects of biomass resources and processing temperatures on the total gas and methane production of the diet are shown in Table 4. It can be seen in the table that biomass resources and processing temperatures affected total gas production (ml/gDM) at 4, 24, and 48 h of incubation ($P < 0.01$). Interactions between biomass resources and processing temperatures affected total gas production at 4 h ($P = 0.06$) and 24 h ($P = 0.001$), but not at 48 h ($P = 0.531$). Biomass resources and processing temperatures affected methane production (ml/gDM) at different incubation times, except for the effect of biomass resources on methane production at 24 h ($P = 0.406$). Interactions between biomass resources and processing temperatures affected methane production at 4 and 48 h ($P < 0.001$), but not at 24 h ($P = 0.096$). The production of gas and methane was higher for corncob-derived biochar than its rice straw and bamboo tree ($P < 0.05$). Increased processing temperature from 300 to 700 °C reduced total gas and methane production at different incubation times ($P < 0.05$). Total gas and methane production was lowest when biochar was produced at 700 °C.

Biomass resources and processing temperatures and their interactions affected the ratio between methane and total gas after 4 h of incubation. However, they did not have any effects after 24 and 48 h of incubation ($P > 0.05$).

3 In vitro *digestibility, pH, and NH_3-N concentration*

The effects of biomass resources, processing temperatures, and their interactions on in vitro digestibility, pH, and NH_3-N concentration (mg/100 ml) are shown in Table 5. In vitro *digestibility of* DM and OM and NH_3-N concentration increased with incubation time. Biomass resources, processing temperatures, and interactions affected the digestibility of DM after 24 and 48 h of incubation ($P < 0.05$). Biomass resources affected OM digestibility after 4 and 24 h of incubation, but it was not the case after 48 h. Processing temperatures and their interaction with biomass resources affected OM digestibility after 48 h of incubation ($P < 0.001$).

Table 4 Effects of biochar produced from tropical rice straw, corncob, and bamboo tree at different processing temperatures on in vitro total gas and methane production at 4, 24, and 48 h after incubation

Items	Biomass sources			Temperature level			SEM	*P*-value		
	R	C	B	300	500	700		Bio	T	Bio×T
Gas production, ml/gDM										
4 h	30.4^{b}	33.7^{a}	30.6^{b}	33.3^{a}	31.3^{b}	30.1^{c}	0.222	<0.001	<0.001	0.060
24 h	141.7^{b}	151.9^{a}	140.6^{b}	151.0^{a}	147.5^{b}	138.8^{c}	0.574	<0.001	<0.001	0.001
48 h	220.5^{b}	231.3^{a}	221.1^{b}	231.5^{a}	226.4^{a}	215.0^{b}	1.416	0.002	<0.001	0.531
CH_4 production, ml/gDM										
4 h	4.17^{ab}	4.30^{a}	4.10^{b}	4.54^{a}	4.25^{b}	3.75^{c}	0.034	0.039	<0.001	<0.001
24 h	21.7	22.5	21.6	23.5^{a}	22.4^{a}	20.6^{b}	0.334	0.406	<0.001	0.096
48 h	30.8^{b}	34.6^{a}	31.3^{b}	34.2^{a}	32.4^{b}	30.1^{c}	0.172	<0.001	<0.001	<0.001

R, rice straw; *C*, corncob; *B*, bamboo tree; *Bio*, biomass resource; *T*, temperature; *Bio*×*T*, interaction between biomass resource and temperature; *SEM*, standard error of mean with $df_{error} = 36$. $^{a–c}$Means within rows and within each factor without a common superscript letter are different at $P < 0.05$

Table 5 Effects of biochar produced from tropical rice straw, corncob, and bamboo tree at different processing temperatures on in vitro digestibility, pH, and NH_3–N concentration

Items	Biomass sources			Temperature level			SEM	*P*-value		
	R	C	B	300	500	700		Bio	T	Bio×T
DM digestibility, %										
4 h	21.5^{a}	20.6^{b}	20.3^{b}	20.5	20.5	21.4	0.119	<0.001	0.051	0.149
24 h	51.7^{a}	50.2^{b}	53.5^{c}	50.8^{c}	52.8^{a}	51.9^{b}	0.144	<0.001	<0.001	0.021
48 h	56.6^{a}	54.3^{b}	55.4^{c}	54.2^{b}	56.1^{a}	56.0^{a}	0.167	<0.001	0.022	0.006
OM digestibility, %										
4 h	22.8^{b}	25.9^{a}	23.0^{b}	24.0	23.8	24.0	0.188	<0.001	0.783	0.781
24 h	54.5^{b}	54.0^{b}	55.7^{a}	55.0	55.5	54.6	0.191	0.001	0.572	0.918
48 h	58.4	58.4	58.4	59.2^{a}	58.8^{b}	57.2^{c}	0.071	0.979	0.001	0.001
pH										
4 h	6.93^{a}	6.81^{b}	6.85^{ab}	6.89^{a}	6.91^{a}	6.79^{b}	0.017	0.008	0.007	0.015
24 h	6.71	6.69	6.68	6.73^{a}	6.70^{a}	6.45^{b}	0.009	0.196	0.002	0.022
48 h	6.66^{a}	6.63^{b}	6.63^{b}	6.69^{a}	6.64^{a}	6.59^{b}	0.005	0.007	<0.001	<0.001
NH_3-N concentration, mg/100 ml										
4 h	5.24	5.26	5.33	5.21	5.28	5.33	0.044	0.645	0.560	0.249
24 h	7.84^{b}	8.24^{a}	7.59^{c}	7.71^{a}	7.88^{a}	8.04^{b}	0.038	<0.001	<0.001	0.054
48 h	8.13^{b}	8.45^{a}	8.03^{c}	8.04^{a}	8.22^{b}	8.33^{c}	0.019	<0.001	<0.001	0.186

R, rice straw; *C*, corn cob; *B*, bamboo tree; *Bio*, biomass resource; *T*, temperature; *Bio*×*T*, interaction between biomass resource and temperature; *SEM*, standard error of mean with $df_{error} = 36$. $^{a–c}$Means within rows and within each factor without a common superscript letter are different at $P < 0.05$

Biomass resources, processing temperatures, and their interactions affected pH at different incubation time, except at 24 h when biomass resources did not affect the pH of incubation. The pH at 4 and 48 h of incubation was higher for rice straw than for corncob and bamboo tree ($P < 0.05$). Increased processing temperatures decreased pH ($P < 0.05$). Biomass resources and processing temperatures independently affected NH_3-N concentration after 24 and 48 h of incubation ($P < 0.001$). NH_3-N concentrations at 24 and 48 h after incubation were higher for corncob compared to rice straw and bamboo tree ($P < 0.05$). Similarly, NH_3-N concentration at 24 and 48 h of incubation was greater for rice straw than a bamboo tree ($P < 0.05$). Increased processing temperatures resulted in higher NH_3-N concentrations at 24 and 48 h of the incubation ($P < 0.05$).

4 Discussion

The production of methane from ruminant livestock should be minimized in order to contribute to sustainable agriculture and the mitigation of climate change. There have been several nutritional strategies to reduce methane production from ruminants. Biochar supplementation to ruminants' diets has attracted significant concerns recently. Supplementing biochar (with and without biochar, different levels

of biochar) has shown to decrease from 5 to 25% methane production, both in vitro and in vivo experiments [7–10]. The different types of biochar used in different experiments were probably the reason for this high variation in methane reduction. Biochar characteristics can vary with biomass resources and pyrolysis procedures, leading to differences in rumen fermentation and gas and methane production [16]. Therefore, we hypothesized that biochars produced from different tropical biomass resources and at different processing temperatures have different characteristics, thus manipulating rumen fermentation and methane production.

Results of this study confirmed our abovementioned hypothesis. Biochar produced at higher temperatures had a larger surface area and a higher water holding capacity. When the processing temperature increased from 300 to 700 °C, the biochar surface area increased from 2.7 to 211.6, 1.1 to 98.6, and 5.4 to 154.2 m^2/g, respectively, for rice straw, corncob, and bamboo tree; water holding capacity increased from 4.8 to 6.6, 3.2 to 5.5, and 3.6 to 5.8, respectively (Table 1). This confirms the findings of Bonelli et al. [17] who reported that increasing pyrolysis temperature causes the increase in biochar surface area and porosity. This is most likely due to the decomposition of organic matter and the formation of micropores, as explained by Katyal et al. [18]. In addition, according to Shaaban et al. [19], a higher pyrolysis temperature causes the release of volatile matter and creates more pores. Moreover, Chen and Chen [20] declared that the destruction of aliphatic alkyls and ester groups, as well as the exposure of the aromatic lignin core under higher pyrolysis temperatures, may result in increased surface area. According to Ghani et al. [21], at lower pyrolysis temperatures, less than 500 °C, lignin is not converted into a hydrophobic polycyclic aromatic hydrocarbon, and biochar becomes more hydrophilic. At higher pyrolysis temperatures, more than 650 °C, biochar is thermally stable and becomes more hydrophobicity.

The increase in processing temperature reduced total gas and methane production at different incubation times (Table 4). This is probably due to increasing the surface area and water holding capacity when biochar is produced at a higher temperature. Biochar with a larger surface area absorbs and adsorbs more gasses and/or methane [5, 9]. In addition, methanotrophic proteobacteria and methanogenic archaea are the key bacteria responsible for methane production. Increasing the methanotrophs group increases methane oxidation, thus reducing methane accumulation [22]. In the rumen, biochar supplementation provides habitat and stimulates methanotrophic growth, thus reducing methane accumulation [6]. Furthermore, biochar produced from high pyrolysis temperature has high electrical conductivity and electron buffering capacity of redox reactions decomposing from the feed [11].

The biomass sources affected the total production of gas and methane. Corncob produced higher total gas and methane production than biochar derived from rice straw and bamboo trees (Table 4). This is probably due to the smaller surface area and water-holding capacity of corncob derived biochar than its rice straw and bamboo tree counterparts. The effects of biomass resources on total gas and methane production were not consistent in the literature. Cabeza et al. [7] reported that biochar prepared from *Miscanthus* reduced total gas and methane to the greatest extent and biochar prepared from rice husk and softwood pellets was least effective. Hansen et al. [8] reported that straw-derived biochar numerically reduced methane to a greater extent than wood-derived biochar. However, Calvelo Pereira et al. [23] did not find any differences in total gas and methane production between wood- and crop residue–derived biochar (i.e., corn stover and pine wood chips). McFarlane et al. [16] found no effects of biomass resources (chestnut, yellow poplar, white pine) on gas production. Calvelo Pereira et al. [23] and Gurwick (2013) found no clear relationships between biochar chemical composition and in vitro total gas and methane production. This may explain for non-effects of biomass resources on total gas and methane production. According to Teoh et al. [24], the variable success rate of previous biochar studies in reproducing significant methane mitigation has been largely attributed to variation in biochar properties such as particle size, adsorptive potential, electrical conductivity, and ability to act as an electron mediator in redox reactions during digestion.

In this study, biomass sources affected the digestibility of DM at 4, 24, and 48 h and the digestibility of OM at 4 and 24 h after incubation. The processing temperature affected the digestibility of DM at 4, 24, and 48 h, and the digestibility of OM at 48 h after incubation. The effects of biochar supplementation in diets on digestibility of DM and OM were not consistent in the literature. Winders et al. [10] could not find differences in DM and OM digestibility between two levels of 0.8 or 3% biochar supplementation. This was also confirmed by Hansen et al. [8]. According to Teoh et al. [24], supplementing with up to 800 mg/day of hardwood biochar over a 15-day period did not affect DM digestibility. Teoh et al. [24] also argued that biochar is 100% inorganic matter and not metabolized by the rumen microbiota. However, Saleem et al. [25] found improved digestibility of DM and OM when biochar was added to diets. They explained that biochar encourages biofilm creation, which stimulates the growth of desirable microbes by providing a niche for their continued proliferation. On the contrary, McFarlance et al. (2017) found reduced DM digestibility when biochar was supplemented with the level of 81 g/kg DM. The authors argued that the

inconsistent findings were due to differences in biomass sources, particle size, and pyrolysis conditions.

pH and NH_3-N are important parameters that regulate rumen fermentation. Therefore, it is important to study the effects of biochar supplementation on the pH and NH_3-N. The literature shows the effects of biochar supplementation and sources on pH and NH_3-N concentration. For example, Zhang et al. [26] declared that biochar supplementation leads to an increased pH value due to the alkaline nature of biochar. Mirheidari et al. [27] reported a stable of ruminal pH due to the lack of changes in primary ruminal fluid VFA, acetic and propionic concentrations among treatments of 0 (no added biochar, control), 1% walnut shell biochar (WSB), 1% pistachio by-product biochar (PBB), and 1.5% chicken manure biochar (CMB). Cabeza et al. [7] reported a reduction in ruminal pH and NH_3-N concentration when biochar was prepared from Miscanthus straw, oilseed rape straw, and softwood pellets at 1.16% of feed substrate were added to incubations. However, Mirheidari et al. [27] reported that the inclusion of WSB, PBB, and CMB increased ruminal NH_3-N concentration by 34.5, 25.06, and 18.89%, respectively.

In this study, a higher processing temperature decreased pH and increased NH_3-N concentration after 24 and 48 h of incubation. We expected a reduction of NH_3-N concentration when biochar was produced at a higher temperature because of its larger surface area, which can adsorb more NH_3-N. However, it was not the case in this study. This can be explained. Effects of biochar on the pH and NH_3-N concentration also depend on the archaeal and bacterial rumen microbiota, the fungal community structure, and VFA concentration. In this study, these criteria were not measured. Future studies should analyze the rumen archaeal, bacterial, and fungal microbiotas, and VFA concentration. Another speculation is that NH_3-N concentration also depends on (i) proteolysis and deamination of nitrogen constituents in the substrate and (ii) incorporation of NH_3-N into microbial protein or combine the two processes. Increased biomass processing temperature resulted in reduced gas production, which is the energy supply for microbial growth. This may be the reason for the increase in nitrogen deamination to provide energy for microbial growth. This process releases NHtemperatures of 300, 500, and 700 °C contributed to differences in in vitro total gas and methane production, digestibility of dry matter and organic matter, pH and NH_3-N concentration. Total gas and methane production was lowest when biochar was processed at 700 °C compared to their counterparts at lower temperatures of 300 and 500 °C. Total gas and methane production was greater for corncob- than rice straw– and bamboo tree–derived biochars. To reduce gas and methane production, either rice straw or bamboo trees should be processed at 700 °C to produce biochars and supplement in ruminant diets.

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Author contribution Dinh Van Dung and Le Duc Thao: conceptualization, methodology, analysis, writing original draft preparation, writing—review and editing. Le Duc Ngoan and Hynek Roubík: writing—review and editing. Le Dinh Phung: methodology, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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