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Evaluation of biochar products at two inclusion levels on ruminal *in vitro* methane production and fermentation parameters in a Timothy-hay based diet

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Abstract

G.F. Mengistu., McAllister T. A., Tamayao, P. J., Ominski, K. H., Ribeiro Jr, G. O., Okine, E. K., McGeough, E. J. This study evaluated the effects of seven biochar products at two levels of inclusion (2.25 or 4.50 % diet DM) on DM disappearance (DMD), cumulative gas and methane (CH_4) production, ammonia-nitrogen and VFA production from Timothy grass hay over 48 h of incubation. Biochar did not affect gas and CH_4 production ($P \geq 0.17$) nor the DMD or ruminal fermentation ($P \geq 0.12$). In conclusion, the biochar, irrespective of level of inclusion, did not exhibit potential to mitigate CH_4 emission in a grass hay diet.

Key words: Biochar, Grass hay, In vitro, Methane, Digestion

Abbreviations

ADF, acid detergent fiber**;** **CH_4,** methane; **CP,** crude protein**;** **DM,** dry matter; **DMD**, DM disappearance; **NDF,** neutral detergent fiber; **NH_3 -N,** ammonia nitrogen**;** **TDN**, total digestible nutrients; **VFA**, volatile fatty acids

Greenhouse gas mitigation strategies in the cattle industry have long been sought, with a particular interest in reducing enteric methane (CH_4) emissions. High-fiber hay diets such as those utilized in the Canadian cow/calf sector, inherently stimulate enteric CH_4 production through a relative increase in acetic and butyric acid and a reduction in propionate production during the microbial fermentation of feed in the rumen (Jayanegara et al. 2014).

In recent years, the potential of biochar to reduce enteric CH_4 production has been studied because of its proposed ability to adsorb gases and participate in redox reactions (Schmidt et al. 2019). Biochar is a carbon-rich material resulting from pyrolysis of biomass including wood, leaves, hulls, straws and animal excreta (Schmidt et al. 2019). Biochar inclusion (0.6% diet DM)

combined with nitrate increased weight gain, feed conversion and reduced (22%) total CH_4 production (ppm) in southeast Asian cattle (Leng et al. 2012). Similarly, Hansen et al. (2012) reported a CH_4 reduction trend of 11-17% (ml/g DM incubated) when straw and wood based biochar were included at 9% of diet DM to *in vitro* incubations. It is suggested that differences in source material as well as pyrolysis conditions can influence the final composition of biochar (i.e., porosity, carbon content, pH) and its potential effects when included as a dietary additive in animal systems (Leng et al. 2012; Schmidt et al. 2019). However, the CH_4 reducing properties of biochar are not always apparent as reported in several studies *in vitro* (Tamayao et al. 2021) and *in vivo* (Terry et al. 2019) with total mixed rations, with little data existing for its use in forage-only diets. This study evaluated the effects of seven biochar products at two levels of inclusion in a grass hay-based diet on total gas and CH_4 production, feed disappearance and rumen fermentation using a rumen *in vitro* batch culture technique.

Materials and methods

Animal care and handling

The animals used in the study were handled according to the Canadian Council on Animal Care Guidelines (AC10997; CCAC 2009), with experimental procedures approved by the University of Manitoba Animal Care Committee.

Experimental design and treatments

The experiment was a randomized complete block design with three laboratory replicates and three runs per experiment. The biochar products used in this study were pyrolyzed from either

coconut (CP001, CP014) or pine (CP002, CP015, CP016, CP023, CP024) sources (Table 1), provided by Cool Planet® (Greenwood Village, CO, USA). These products differed in chemical and physical characteristics thus providing the basis of evaluating a range of intrinsic biochar properties and sources. Biochar treatments consisted of Timothy grass hay (DM 851; CP 108; NDF 532; ADF 348; TDN 615 g/kg DM) with the products listed above included at two levels (2.25 and 4.50 % diet DM) based on earlier biochar studies (batch culture and RUSITEC) conducted at our institution. A control treatment of grass hay only was also included.

In vitro incubation

The Timothy grass hay and biochar products were oven-dried at 55°C for 48 h and ground through a 1 mm screen (Wiley Mill Thomas Scientific., Swedesboro, NJ, USA). Timothy grass hay (0.5 g DM), with respective biochar and level of inclusion, was weighed into ANKOM bags (F57, Ankom Technology®, Macedon, NY, USA) washed with acetone before use and placed in incubation vials (120 mL). Buffer mineral solution was prepared at 39°C, continuously flushed with CO_2. Rumen fluid was collected 2 h post-feeding from three ruminally cannulated beef heifers offered Timothy hay only, ad libitum daily 14 d prior to the experiment. Rumen fluid was strained through a Pecap mesh (mesh size 250 µm; PA66CG-250 136 cm, Sefar Nytal, Gilbert Saguenay, QC, CA) and then composited and re-filtered through three layers of cheese cloth, with CO_2 continuously flushed to maintain anaerobic conditions. The inoculum (15 ml rumen fluid:45 ml buffer mineral solution; Menke et al. 1979), was dispensed into each vial which was then placed on an orbital shaker (speed at 60 rpm, TYZD-III orbital shaker; Jiangsu Tenlin Instrument, Jiangyan, China) inside an incubator at 39°C (VWR Scientific, Model 2020, Mississauga, ON, CA) and incubated for 48 h.

Measurements

Sample bags were removed from each bottle, rinsed with distilled water and placed in a forced air-drying oven at 55°C for 48 h. The DMD was determined (expressed as percent digestibility) as the difference between the substrate DM incubated and dry weights of sample residues after incubation divided by the substrate DM incubated multiplied to 100. Gas samples were collected and injected (syringe, 25-gauge ½ needle) into gas exetainers (6.8 mL; Labco, Ltd., Wycombe, London, UK) at 3, 6, 9, 12 18, 36 and 48 h and gas pressures were recorded at all time-points using a pressure transducer (Traceable pressure calibrator, model 33500-086, Friendswood, TX, USA), thereafter gases were vented from each vial. Cumulative gas pressure measurements obtained from each time point were used to calculate total gas production (GP; Equation 3; Romero-Perez and Beauchemin 2018). Methane concentration was determined from gas samples obtained at each timepoint via gas chromatography (Agilent 7890B series GC custom, Agilent Technologies Canada Inc., Mississauga, ON, CA) and totaled values from all time points expressed as mL/g DM (incubated) and mL/g DMD. Analysis of VFA was completed as per the techniques of Erwin et al. (1961), where 3 mL of the fermented fluid from each vial was placed into pre-filled tubes containing 25% metaphosphoric acid (0.6 mL). Total VFA and molar proportions of individual acids and acetic to propionic (A:P) ratio were determined. For NH_3-N analysis, 3 mL of fermented fluid was collected from each vial into pre-filled tubes containing 7.2 N sulfuric acid (0.6 mL) and were prepared using the Indole-phenol method (Novosamsky et al. 1974) with concentrations determined via UV spectrophotometer (absorbance measured at 655 nm; Ultrospec 3100 pro UV/Visible, Cambridge, England, UK).

Statistical analysis

Data were analyzed via PROC MIXED procedure of SAS 9.4 (SAS 2018). Fixed factors included biochar products, inclusion level and interactions, with run and replicate within run included as random factors. Within each experimental run were three vials per treatment and were randomly placed in a three-level incubator, with one treatment vial randomly assigned in each level to account for any incubator gradient effect. Each level inside the incubator was considered a replication. Variance components was used as the covariance structure for the analysis of all DMD, gas and fermentation parameters. Means were separated using Tukey-Kramer's multiple range test at $P < 0.05$. No significant interactions occurred thus contrasts were not performed.

Results

Biochar source and level of inclusion did not affect DMD of grass hay (P=0.50; Table 2). For GP, the control did not differ ($P \geq 0.56$) from biochar treatments, regardless of source or level of inclusion. Methane production was not affected ($P \geq 0.17$) by biochar source nor level of inclusion irrespective of unit of expression. Rumen fermentation was not affected by biochar source nor level of inclusion, as no differences in total VFA (P=0.38) or individual molar proportions of acetic, propionic, butyric and valeric acid ($P \geq 0.12$) were observed. Additionally, A:P ratio and NH_3-N were unaffected ($P \geq 0.15$).

Discussion

Although data exists evaluating biochar supplementation in TMR and high grain diets, few studies have evaluated it in starch free, hay-based diets. Supplementation of biochar to a Timothy-

hay based diet did not impact feed disappearance, regardless of biochar source or level of inclusion. This is consistent with the findings of Terry et al. (2019) and Tamayao et al. (2021). This may be due to the recalcitrant nature of biochar as it is not degraded or metabolized in the rumen (Schmidt et al. 2019). Similar to the current study, the reported lack of significant responses in both gas and methane production were consistent with the lack of differences in substrate DM disappearance. Regardless of the range and differences in physical (bulk density, surface area) and chemical (pH, carbon content) properties, biochar did not have an impact on ruminal gas production and fermentation, likely due to the indigestible nature of biochar. This is reflective of the DMD results as the substrate influences gas, CH_4 and the resultant by-products of rumen fermentation (Tamayao et al. 2021). However, Leng et al (2012) reported a 22 % reduction in total CH_4 (ppm) production though this was likely reflective of the increased weight gain and feed conversion efficiency in South East Asian cattle. These authors hypothesized that the porous nature of biochar facilitated redox reaction between bacterial species and favored the proliferation of rumen biofilm microbiota (and possibly rumen methanotrophs) with biochar pores serving as microhabitats which resulted in increased feed conversion efficiency; ultimately, this mechanism may decrease the CH_4 output per unit of feed. This, however, is unlikely as low levels (0.5 - 1.0 % diet DM) of biochar would not have the capacity to mediate redox reactions in cattle given the quantity of CH_4 produced per animal (Schmidt et al. 2019) and electron microscopy suggests that biofilms on biochar surfaces are less developed than those on more readily digested substrates (Terry et al. 2019). This was further supported by the metagenomic analysis findings of Tamayao et al. (2021) as rumen microbial families were not affected by biochar, resulting in the lack of ruminal gas and fermentation responses. The observed reductions in CH_4 production observed by Leng et al. (2012) may also have been due to the combined use of nitrate, as reduction in CH_4 following nitrate

supplementation is well established (Schmidt et al. 2019). Notwithstanding, biochar effects on rumen CH_4 production are inconsistent and may also be attributed to other factors such as the physiochemical properties of biochar influenced by source of origin and pyrolysis conditions (Schmidt et al. 2019) and inherent rumen microbiota present in the animals (Terry et al. 2019). Overall, biochar did not demonstrate potential to mitigate ruminal CH_4 production in a grass hay-based diet.

Conclusions

The inclusion of seven biochar products differing in source and level of inclusion did not affect *in vitro* ruminal DMD or rumen fermentation and did not offer CH_4 mitigation potential for Timothy hay diets.

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References

CCAC. 2009. Guide to the care and use of farm animals in research, teaching and testing. **Canadian Council on Animal Care**, Ottawa, ON.

Erwin, E. S., Marco, G. J., Emery, and E. M. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. **44**: 1768-1771. https://doi.org/10.3168/jds.s0022-0302(61)89956-6.

Hansen, H.H., Storm, I. M. L. D. and Sell, A. M. 2012. Effect of biochar on in vitro rumen methane production. Acta Agr. Scan. **62**: 305-309. https://doi.org/10.1080/09064702.2013.789548.

Jayanegara, A., Wina, E., and Takahashi, J. 2014. Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: influence of addition levels and plant Sources. Asian-Australas. J. Anim. Sci. **27(10)**: 1426–1435. http://doi.org/10.5713/ajas.2014.14086.

Leng, R. A., Inthapanya, S. and Preston, T. R. 2012. Methane production is reduced in an in vitro incubation when the rumen fluid is taken from cattle that previously received biochar in their diet. Livestock Res. Rural Develop. **24**: 211. [Online] Available: https://www.lrrd.org/lrrd24/11/sang24211.htm.

Menke, K.H., Raab, L., Salewski, A., Steingrass, H., Fritz, D., and Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agr. Sci. **93**:217. https://doi.org/10.1017/S002 1859600086305.

Novosamsky, I. R., Van Eck, J. C., Showenburg, H., and Walinga, F. 1974. Total nitrogen determination in plant material by means of the indole-phenol blue method. Neth. J. Agri. Sci. **22**:3-5.

Romero-Pérez, A., and Beauchemin, K. A. 2018. Estimating gas volume from headspace pressure in a batch culture system. Can. J. Anim. Sci. **98(3)**: 593-596. https://doi.org/10.1139/cjas-2017-0100.

SAS, 2018. Statistical Analysis Software. SAS University Edition.User's guide Cary, N.C.

Schmidt, H. P., Hagemann, N., Draper, K., and Kammann, C. 2019. The use of biochar in animal feeding. Peer J. **7**: e7373. https://doi.org/10.7717/peerj.7373.

Tamayao, P. J., Ribeiro, G. O., McAllister, T. A., Yang, H. E., Saleem, A. M., Ominski, K. H., Okine, E. K. and McGeough, E. J., 2021. Effects of post-pyrolysis treated biochars on methane production, ruminal fermentation, and rumen microbiota of a silage-based diet in an artificial rumen system (RUSITEC). Anim. Feed Sci. Technol. **273**:114802. https://doi.org/10.1016/j.anifeedsci.2020.114802.

Terry, S.A., Ribeiro, G.O., Gruninger, R.J., Chaves, A. V., Beauchemin, K.A., Okine, E. and McAllister, T.A. 2019. A Pine Enhanced Biochar Does Not Decrease Enteric CH_4 Emissions, but Alters the Rumen Microbiota. Front. Vet. Sci. **6**: 1–12. https://doi.org/10.3389/fvets.2019.00308.

Table 1. Biochar source and physiochemical characteristics

Item	Biochar product						
	CP001	CP002	CP014	CP015	CP016	CP023	CP024
Source/biomass origin	Coconut	Pine	Coconut	Pine	Pine	Pine	Pine
Chemical characteristics							
Carbon, % DM	75.6	81.6	76.6	75.4	76.9	75.3	71.1
pH	6.3	5.8	5.0	4.9	4.9	7.6	7.3
Physical characteristics							
Bulk density, kg/m^3	706.0	310.0	606.0	262.0	287.0	122.0	140.0
Surface area, m^2/g	161.0	218.0	160.0	189.0	186.0	152.0	148.0
Pore volume, cc/g	6.45×10^{-2}	8.75×10^{-2}	6.52×10^{-2}	7.56×10^{-2}	7.36×10^{-2}	6.10×10^{-2}	6.00×10^{-2}
Particle size distribution, mm							
D 0.1	0.25	0.15	0.29	0.29	1.60	0.85	0.73
D 0.5	0.95	1.25	0.47	0.50	4.30	1.95	1.75
D 0.9	1.85	2.95	0.73	0.83	5.95	3.00	3.15

Table 2. Effects of biochar product and inclusion level on *in vitro* DMD, gas and CH_4 production and rumen fermentation parameters in a grass hay-based diet

	Biochar product																
	Control	CP001		CP002		CP014		CP015		CP016		CP023		CP024		SEM	*P*-value
Inclusion level, % diet DM	0	2.25	4.50	2.25	4.50	2.25	4.50	2.25	4.50	2.25	4.50	2.25	4.50	2.25	4.50		
Parameter																	
DMD, %	44.4	43.1	41.1	41.1	43.2	41.6	43.3	42.2	44.1	42.5	43.2	43.6	42.7	41.5	42.7	2.11	0.50
Gas and CH_4 production																	
GP[z], ml/g DM	135.8	136.2	133.8	138.6	129.6	135.7	129.7	139.2	131.4	134.5	134.5	133.8	137.0	134.8	129.1	8.21	0.85
GP, ml/g DMD	156.4	165.9	171.3	166.0	169.0	164.3	166.6	163.9	166.3	160.6	167.9	165.2	170.7	166.0	164.5	7.90	0.56
CH_4, ml/g DM	16.5	17.3	17.7	15.7	17.9	15.8	17.4	15.9	18.8	16.8	17.1	18.1	17.5	17.4	18.8	2.47	0.33
CH_4, ml/g DMD	25.7	28.4	25.8	26.7	25.8	26.4	25.2	25.3	25.8	25.8	23.16	26.1	26.3	25.7	26.4	2.16	0.17
Rumen fermentation																	
Total VFA, mmol/L	33.3	32.6	28.7	30.7	39.3	32.4	37.1	33.4	28.7	27.1	39.0	23.2	36.4	28.0	35.9	5.86	0.38
Acetic	22.2	24.0	24.5	23.0	25.5	22.7	26.1	23.4	24.9	23.1	26.3	22.1	22.1	23.3	27.8	3.39	0.17
Propionic	9.4	8.6	9.0	8.9	10.0	8.0	10.1	8.8	9.1	8.0	10.1	8.3	9.2	9.3	9.7	0.77	0.26
Butyric	4.7	5.4	4.6	4.7	5.7	4.2	5.2	5.1	4.8	3.9	5.3	3.8	4.8	4.5	4.8	0.54	0.12
Valeric	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.4	0.06	0.42
Acetic:Propionic	2.5	2.6	2.7	2.6	2.6	2.7	2.7	2.6	2.8	2.8	2.6	2.6	2.5	2.6	2.8	0.24	0.60
NH_3-N, mg/dL	5.2	5.0	5.0	4.9	4.5	5.3	5.3	4.7	4.8	5.2	5.0	5.1	5.5	4.3	5.6	0.64	0.15

[z]GP: Gas production