Evaluation of the effects of biochar on diet digestibility and methane production from growing and finishing steers

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ABSTRACT: The objectives of these studies were to evaluate the effects of biochar (0%, 0.8%, or 3% of diet dry matter) on diet digestibility and methane and carbon dioxide production from cattle on growing and finishing diets. The growing diet consisted of 21% brome hay, 20% wheat straw, 30% corn silage, 22% wet distillers grains plus solubles, and 7% supplement. The finishing diet consisted of 53% dry-rolled corn, 15% corn silage, 25% wet distillers grains plus solubles, and 7% supplement. In both trials biochar replaced fine ground corn in the supplement. Six crossbred steers (initial body weight [BW] 529 kg; SD = 16 kg) were used in both the growing and finishing trial. The growing diets were evaluated over 6 periods followed by the finishing trial with 3 periods. Digestibility measures were taken over 4 d after at least 8 d of adaptation to diets followed by 2 d of gas emission measurements using headbox calorimeters. Dry matter intake (DMI) was not affected ($P \geq 0.43$; 7.91 kg/d) by biochar inclusion in the growing study and increased quadratically ($P = 0.07$) in the finishing study with 0.8% biochar inclusion having the greatest DMI (12.9 kg/d). Organic matter (OM) and neutral detergent fiber (NDF) digestibility increased quadratically ($P = 0.10$) in the growing study whereas OM digestibility tended to linearly decrease ($P = 0.13$) and NDF digestibility was not affected ($P \geq 0.39$) by biochar inclusion in the finishing diet. Digestible energy intake (Mcal/d) was not affected ($P \geq 0.25$) by biochar inclusion in the growing or finishing study. Methane production (g/d) tended to decrease quadratically ($P = 0.14$) in the growing study and was decreased 10.7% for the 0.8% biochar treatment relative to the control. There were no statistical differences in methane production (g/d) in the finishing study ($P \geq 0.32$) but cattle on the 0.8% biochar treatment produced numerically less (9.6%) methane than the control. Methane production as g/kg DMI of the 0.8% biochar treatment relative to the control was numerically reduced 9.5% and 18.4% in the growing and finishing studies, respectively ($P \geq 0.13$). Carbon dioxide production (g/d and g/kg of intake) quadratically decreased ($P \leq 0.06$) in the growing study but was not affected by treatment in the finishing study ($P \geq 0.34$). Although biochar is not a U.S. Food and Drug Administration -approved feed for cattle, the initial research shows potential as a methane mitigation strategy in both growing and finishing diets.

Key words: beef cattle, biochar, digestibility, methane

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INTRODUCTION

Energy lost as methane (CH_4) by ruminants can range from 2% to 12% of total gross energy

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intake (GEI), but is variable depending on diet composition and energy density (Johnson and Johnson, 1995). Production of CH_4 is a necessary component of rumen fermentation, but is an energy sink to the animal and has been implicated in global warming (Boadi et al., 2004).

Biochar is produced by burning organic matter (OM; typically plant material) in the absence of oxygen (Hansen et al., 2012). Although biochars' mode of action is not fully understood, suggested mechanisms include biochar adsorbing gas in the rumen resulting in reduced CH_4 eructation, the porous nature of biochar increasing inert surface area in the rumen allowing for improved microbial habitat, or altering the microbial community (Leng, 2014; Saleem et al., 2018). Feng et al. (2012) found that biochar increases the ratio of methanotrophs to methanogens in paddy soils, and this process may also occur in the rumen. Feeding biochar has been shown to decrease production of CH_4 from in vitro systems for hay (Hansen et al., 2012), cassava root meal–based diets (Leng et al., 2012b), and barley silage diets (Saleem et al., 2018). However, the feedstock and process used to produce the biochar may affect results (Leng et al., 2013; McFarlane et al., 2017). In vivo results of feeding biochar to cattle are limited, Leng et al. (2012a) reported a decrease in CH_4 production from cattle fed diets based on cassava root chips and foliage whereas Erickson et al. (2011) measured an increase in diet digestibility when activated carbon was added to poor quality corn silage diets. The objectives of the following experiments were to determine the effects of biochar on CH_4 production and diet digestibility in vivo in growing and finishing beef cattle diets composed of feeds commonly used in the Great Plains of the United States.

MATERIALS AND METHODS

All animal care and management practices were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee (approval number 1282). Because biochar is not currently approved by the U.S. Food and Drug Administration to be fed to cattle entering the human food chain, all cattle were killed under veterinary supervision and composted at completion of the experiments.

Growing Experiment

An indirect calorimetry study evaluated diet digestibility and CH_4 production for growing cattle fed varying inclusions of biochar (High Plains Biochar LLC, Laramie, WY). Biochar was made from whole pine trees, including limbs and needles, using commercial biochar equipment (BioChar King BK 1000; OrganiLock, Inc., Madisonville, KY). Biochar was analyzed for dioxin and furan contaminants using method 1613B (US EPA, 2010; Pace Analytical, Minneapolis, MN), and the presence of polychloro dibenzo-*p*-dioxins and polychloro dibenzofurans was non-detectable with detection minimums of 1 to 10 ng/kg. Method 6010C (US EPA, 2000) was used to measure concentration of cadmium, lead, and arsenic in the biochar, which were all non-detectable with detection minimums of 0.15, 0.49, and 0.98 mg/kg, respectively. Method 7471B (US EPA, 1998) was used to measure concentration of mercury, which was also non-detectable with a detection minimum of 0.02 mg/kg. The biochar had a composition of 85% carbon, 0.7% nitrogen, and 94% OM on a dry matter (DM) basis with a pH of 8.0. Particle size distribution was 1.0% greater than 9.5 mm, 18.7% 3.35 to 9.5 mm, 44.0% 1.18 to 3.35 mm, 10.8% 0.850 to 1.18 mm, 6.8% 0.600 to 0.850 mm, and 18.7% less than 0.600 mm.

Six crossbred steers (initial body weight [BW] 529 kg; SD = 16 kg) were used in a 6 period repeated switchback design (Cochran and Cox, 1957). Steers were assigned randomly to one of three treatments which alternated over 6 periods; thus, measurements were collected on each animal consuming each treatment during two nonconsecutive experimental periods. Diets fed were identical between treatments other than inclusion of biochar, which displaced fine ground corn in the supplement at 0%, 0.8%, or 3% of diet DM (Table 1). Periods ranged from 14 to 24 d with two consecutive, 23-h periods in a headbox calorimeter. Periods 1, 2, 5, and 6 were 14 d and periods 3 and 4 were 24 and 21 d, respectively. Availability of the calorimeters dictated period length. Each period consisted of adaptation to treatments (minimum of 8 d), fecal grab sampling 4 times/d (0700, 1100, 1500, and 1900 h) on four consecutive d leading up to headbox collections, and headbox collections for the final 2 d of the period. Individual feed ingredient samples were taken weekly and frozen (−4 °C) until trial completion.

Diets were mixed twice weekly in a stationary ribbon mixer (model HD-5, Davis Precision Horizontal Batch Mixer; H.C Davis Sons Manufacturing Co., Inc., Bonner Springs, KS) and stored in 200 L barrels. The barrels were stored in a cooler held at 4 °C to ensure diet quality was maintained. Cattle were fed ad libitum twice daily

Table 1. Composition of diet (DM basis) fed to cattle (growing experiment)

	Biochar inclusion, % DM		
Ingredient, % of diet DM	0	0.8	3
Brome hay	21	21	21
Wheat straw	20	20	20
Corn silage	30	30	30
Wet distillers grains plus solubles	22	22	22
Supplement[1]			
Fine ground corn	4.630	3.830	1.630
Biochar	-	0.800	3.000
Limestone	1.320	1.320	1.320
Tallow	0.175	0.175	0.175
Urea	0.500	0.500	0.500
Salt	0.300	0.300	0.300
Beef trace mineral[2]	0.050	0.050	0.050
Vitamin A-D-E[3]	0.015	0.015	0.015
Rumensin-90[4]	0.010	0.010	0.010
Nutrient analysis, %[5]			
DM	62.1	62.5	62.7
OM	90.6	90.9	90.9
CP	13.5	13.4	13.3
NDF	52.9	53.3	54.6
ADF	35.4	35.8	37.5

CP = crude protein.

[1]Supplement fed at 7% of diet DM.

[2]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

[3]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per gram.

[4]Formulated to supply Rumensin-90 (Elanco Animal Health,; Greenfield, IN) at 20 mg/kg of DM.

[5]Nutrient analysis was measured on weekly grab samples of individual feeds, composited into period samples.

at 0800 and 1500 h. Steers were individually housed in 1.5 × 2.4 m slatted floor pens with rubber mats in a temperature-controlled room (25 °C) and had ad libitum access to water. Feed refusals were weighed back daily and adjustments for feed offered were made accordingly. Feed refusals were weighed, subsampled, and dried at 60 °C for DM determination during the fecal collection period. Fecal samples were composited by day, freeze-dried, and ground to 1 mm using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). The ground samples were then composited by period for each steer. Feed samples were also composited by period, freeze-dried and ground to 1 mm. Feed and fecal samples, composited by period, were dried at 100 °C for 24 h to determine DM and then burned in a cool muffle furnace at 600 °C for 6 h to determine OM.

Feed and fecal samples were also analyzed for neutral detergent fiber (NDF) using the Van Soest et al. (1991) method. Sodium sulfite (0.5 g; Fisher Scientific, Fair Lawn, NJ) was added to the samples before 100 mL of ND solution (Midland Scientific, Davenport, IA) was added. Alpha-amylase (ANKOM Technology, Macedon, NY) was added at the beginning of boiling and at 30 min of reflux in 0.5 mL increments to all fecal, corn silage, wet distillers grains plus solubles, and supplement samples. Feed and fecal samples were analyzed for acid detergent fiber (ADF) using method 973.18 (AOAC International, 2000).

Acid insoluble ash was used as an internal marker to estimate fecal output and diet digestibility. Acid insoluble ash was determined by placing the dried ADF sample into a cool muffle furnace at 600 °C for 6 h. Fecal output was calculated by dividing acid insoluble ash intake by acid insoluble ash in the feces. Acid insoluble ash analysis was done on the base diet fed, feed refusals, and fecal samples to determine acid insoluble ash intake and fecal output, which was used to determine digestibility. Gross heat energy was determined for feed and fecal samples using a Parr 6400 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). Digestible energy was then calculated by subtracting total gross fecal energy from total GEI.

Gas Emissions

CH_4 emissions were measured through indirect calorimetry using headboxes built at the University of Nebraska–Lincoln. Three headboxes were available, so timing of measurements was staggered, with each treatment represented during each collection period. Collections consisted of 2 consecutive, 23 h periods on the final 2 d of each period. The collection method was similar to that described by Foth et al. (2015). A training period of 2 wk was used prior to the experiment in order for steers to become acclimated to the headboxes, with a gradual increase in amount of time spent in the headboxes. One steer was removed from the gas emissions portion of the trial after period 2 because of a lack of dry matter intake (DMI) while in the headbox. Feed was offered ad libitum while the steers were in the headboxes and was adjusted based off refusals throughout the collection period. Feed was placed in the headbox when the steers entered at 0800 h. The doors were then closed and the vacuum motor (Model 115923; Ametek Lamb Electric, Kent, OH) was turned on, creating a negative pressure system in the headbox. Total airflow through the headbox was measured using a gas meter (Model AL425; American Meter, Horsham, PA), and was regulated by flow meters (Model 1350E Sho-Rate 50; Brooks Instruments, Hatfield, PA) to allow for proportional

samples to be gathered. The headbox doors were closed 15 min prior to collection starting to allow for several air turnovers before emissions were collected. The samples were collected in foil bags that continuously and evenly filled throughout the 23-h collection period. Two bags per headbox were continuously filled over the 23-h collection, one bag for ambient air entering the headbox and one for emissions leaving the headbox. Air was diverted to each bag using glass tube rotameters (Model 1350E Sho-Rate "50"; Brooks Instruments). These bags were analyzed for CH_4 and carbon dioxide (CO_2) using a gas chromatograph (Universal Analyzers Inc., Carson City, NV).

After the 23-h collection period, steers were brought back to their pens for 1 h while feed refusals were collected, rubber mats and waterers were cleaned, foil bags switched out, and flow rates were recorded. A second 23-h collection period then followed. Gas measurements collected over the 2 d were averaged to obtain one value per period for each steer. Intakes decreased 12% on average and become more variable when cattle entered the headboxes compared to the 5 d prior to being in the headboxes. Most of the decrease in intake was on d 2 of the headbox period. Therefore, average DMI for the 5 d directly prior to the 2 d headbox period was used to report gas emissions on a grams per kilogram of DMI basis.

Finishing Experiment

The same six steers were then used in a 3-period crossover design with a finishing diet. Steers remained in the same BW block and were assigned randomly within block to one of three treatments. Similar to the growing experiment, diets fed were identical between treatments other than inclusion of biochar (0%, 0.8%, or 3% of diet DM), which displaced fine-ground corn in the supplement (Table 2). Periods were 16 d with two consecutive 23-h headbox collections over the last 4 d of each period. Because three headboxes were available, headbox collections were done over 4 d (six total animals for 2 d each), each treatment was represented in each headbox collection period. Fecal output and diet digestibility were calculated by dosing 10 g/d of titanium dioxide in the feed. Feed and fecal sampling and nutrient analysis were all conducted the same as for the growing experiment, with the exception of titanium dioxide instead of acid insoluble ash as the marker to determine diet digestibility. Titanium dioxide analysis on feed and fecal samples was done using methodology from Myers et al. (2004). Gas emissions were also collected as described in the growing experiment, with all six animals being used.

Table 2. Composition of diet (DM basis) fed to cattle (finishing experiment)

	Biochar inclusion, % DM		
Ingredient, % of diet DM	0	0.8	3
Dry-rolled corn	53	53	53
Corn silage	15	15	15
Wet distillers grains plus solubles	25	25	25
Supplement[1]			
Fine ground corn	4.630	3.830	1.630
Biochar	-	0.800	3.000
Limestone	1.320	1.320	1.320
Tallow	0.175	0.175	0.175
Urea	0.500	0.500	0.500
Salt	0.300	0.300	0.300
Beef trace mineral[2]	0.050	0.050	0.050
Vitamin A-D-E[3]	0.015	0.015	0.015
Rumensin-90[4]	0.010	0.010	0.010
Nutrient analysis, %[5]			
DM	66.9	67.3	67.5
OM	85.4	85.4	85.2
CP	13.3	13.2	13.1
NDF	25.2	25.9	27.9
ADF	10.7	11.2	12.6

CP = crude protein.

[1]Supplement fed at 7% of diet DM.

[2]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

[3]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E. per gram.

[4]Formulated to supply Rumensin-90 (Elanco Animal Health) at 20 mg/kg of DM.

[5]Nutrient analysis was measured on weekly grab samples of individual feeds, composited into period samples.

Statistical Analysis

Statistical analysis was done using the MIXED procedure of SAS (SAS Inst Inc., Cary, NC) for DM digestibility (DMD) as a 6 × 6 balanced replicated Latin rectangle and gas production as an unbalanced replicated Latin rectangle (due to removal of one steer) for the growing experiment and as a 6 × 3 balanced Latin rectangle for the finishing experiment. The model included treatment and period as fixed effects for digestibility and gas production analysis. Steer was considered a random effect in both analyses. Orthogonal contrasts were used to detect linear and quadratic relationships for the main effect of biochar inclusion. Because treatments were not evenly spaced, the IML procedure of SAS was used to generate coefficients used for contrast statements. Biochar included vs. biochar

absent from the diet (i.e. combining the 0.8% and 3% treatments) was also analyzed as a preplanned contrast. Probabilities were considered significant at $P < 0.10$ and tendencies are discussed at $P \leq 0.15$.

RESULTS AND DISCUSSION

Growing Experiment

Digestibility and energy. DMI (kg/d) did not differ between treatments ($P \geq 0.43$; Table 3), but did increase between periods as a result of the cattle growing, and therefore eating more. This is similar to results reported by Leng et al. (2012a) in which authors fed biochar derived from rice husks to cattle in Laos. These authors conducted a 98-d trial feeding biochar at 0.6% of the diet DM in a cassava root chip and cassava foliage–based diet. No differences in DMI were detected, and the authors observed an increase in average daily gain and feed efficiency, but did not report any digestibility measures for the diets fed.

All intake, fecal output and digestibility data are reported in Table 3. A quadratic increase ($P = 0.10$) was observed for OM digestibility (OMD) with the 0.8% biochar treatment having the greatest OMD (60.6%). Similarly, DMD tended ($P = 0.11$) to increase quadratically. A linear decrease ($P = 0.08$) was observed for NDF digestibility (NDFD) with 3% inclusion of biochar having the lowest digestibility (48.2%). GEI (Mcal/d) and digestible energy intake (DEI; Mcal/d) did not differ between treatments ($P \geq 0.27$); however, DEI as Mcal/kg of DMI had a quadratic increase ($P = 0.08$) with 0.8% inclusion of biochar being the greatest at 2.68 Mcal/kg DMI. A tendency was observed for a linear increase in NDF excretion ($P = 0.14$) and ADF intake ($P = 0.13$), whereas energy excreted (Mcal/d) tended to decrease quadratically ($P = 0.13$).

Table 3. Effects of biochar inclusion in cattle diets on intake and total tract digestibility (growing experiment)

	Biochar inclusion, % DM				*P*-values[1]	
Item	0	0.8	3	SEM	Lin	Quad
DM						
Intake, kg/d	8.01	7.88	7.83	0.21	0.43	0.64
Excreted, kg/d	3.57	3.35	3.57	0.16	0.71	0.18
Digestibility, %	55.7	57.6	54.7	1.12	0.25	0.11
OM						
Intake, kg/d	7.25	7.16	7.12	0.19	0.52	0.74
Excreted, kg/d	3.02	2.83	3.03	0.14	0.68	0.18
Digestibility, %	58.6	60.6	57.7	1.16	0.31	0.10
NDF						
Intake, kg/d	4.24	4.19	4.28	0.11	0.62	0.57
Excreted, kg/d	2.11	2.00	2.24	0.11	0.14	0.16
Digestibility, %	50.5	52.6	48.2	1.55	0.08	0.10
ADF						
Intake, kg/d	2.83	2.82	2.93	0.08	0.13	0.53
Excreted, kg/d	1.52	1.47	1.63	0.08	0.16	0.33
Digestibility, %	46.7	48.1	45.0	1.50	0.29	0.35
Energy						
GEI, Mcal/d	35.3	34.8	34.8	0.93	0.62	0.68
Fecal Energy, Mcal/d	14.8	13.8	14.8	0.68	0.67	0.13
DEI, Mcal/d	20.5	21.0	20.0	0.51	0.27	0.30
DEI, Mcal/kg DMI	2.57	2.68	2.56	0.05	0.52	0.08

[1]Linear and quadratic orthogonal polynomial contrasts.

Van et al. (2006) fed a charcoal product derived from bamboo to goats on an acacia foliage and para grass–based diet in Vietnam at inclusions of 0, 1, and 1.5 g per kg of BW. These authors reported that bamboo charcoal did not affect DMI, and improved DMD and OMD values for the 0.5 and 1 g/kg BW treatments compared to the control and 1.5 g/kg BW treatment. The authors attributed the digestibility improvements to the ability of the charcoal to adsorb toxins and tannins, preventing them from reaching the intestines and inhibiting enzyme excretion, resulting in more digestion. However, Kutlu et al. (2001) reported that wood-based biochar products are capable of adsorbing vitamins, fats, and enzymes when included at a high level in poultry diets, which could explain some of the digestibility responses observed in the present trial for the 3% biochar treatment. Saleem et al. (2018) reported a linear increase in DM, OM, crude protein, ADF, and NDFD with the inclusion of 0%, 0.5%, 1%, and 2% biochar to a forage-based (60% barley silage) diet using an artificial rumen system.

CH_4 and CO_2 production. Reported DMI (kg/d) used for gas emission calculations was a 5 d average prior to cattle entering the headboxes, and was not different between treatments ($P \geq 0.68$; Table 4). The GEI and DEI (Mcal/d) based on the 5 d intakes were also not different ($P \geq 0.32$). CH_4 production (g/d) tended ($P = 0.14$) to decrease quadratically with the 0.8% biochar treatment having the lowest CH_4 output at 97.2 g/d. When combining the two treatments that contained biochar (0.8% and 3%) into one to compare to the 0% treatment, CH_4 production (g/d) tended ($P = 0.11$) to be lower for the biochar cattle relative to the control cattle. Saleem et al. (2018) also reported a quadratic response for CH_4 production (mg/d and g/g of DM incubated) with 0.5% biochar having the least CH_4 production. In the current study, the 0.8% biochar treatment reduced CH_4 (g/d) by 11% compared to the control treatment

Table 4. Effects of increasing inclusion of biochar on CH_4 and CO_2 emissions from steers (growing experiment)

	Biochar inclusion, % DM				*P*-values[1]		
	0	0.8	3	SEM	Lin	Quad	Y/N
DMI, kg/d	7.91	7.90	7.84	0.21	0.68	0.90	0.70
GEI, Mcal/d	34.9	34.7	34.8	0.94	0.99	0.85	0.88
DEI, Mcal/d	20.6	21.1	20.3	0.53	0.50	0.32	0.82
CH_4							
g/d	109	97.2	100	5.1	0.42	0.14	0.11
g/kg DMI	13.7	12.4	12.7	0.60	0.43	0.18	0.13
g/Mcal	3.10	2.80	2.86	0.13	0.37	0.17	0.11
GEI							
g/Mcal DEI	5.27	4.62	4.92	0.21	0.51	0.05	0.07
CO_2							
g/d	5549	5051	5163	172	0.19	0.05	0.02
g/kg DMI	702	644	660	18.1	0.27	0.06	0.03
CH_4:CO_2	0.020	0.019	0.019	0.001	0.67	0.70	0.56

[1]Linear and quadratic orthogonal polynomial contrasts. Y/N = biochar inclusion in diet (0.8% and 3% treatments combined) vs. no biochar in diet (0 treatment).

without biochar. This is a smaller response than Leng et al. (2012a) reported with a 24% reduction in CH_4 (ppm) when feeding biochar derived from rice hulls at 0.6% of the diet DM. Similarly, Saleem et al. (2018) reported a 25% reduction in CH_4 (mg/d) from an artificial rumen system with 0.5% biochar compared to no biochar.

CH_4 production measured as g/kg DMI was not different between treatments in the present study ($P \geq 0.18$). When analyzing CH_4 produced per Mcal of GEI, no differences were observed between treatments ($P \geq 0.17$); however, CH_4 per Mcal of DEI was lowest for 0.8% biochar (4.62 g/Mcal DEI) and greatest for the 0% treatment (5.27 g/Mcal DEI), resulting in a quadratic response ($P = 0.05$). When combining treatments, CH_4 as g/kg DMI ($P = 0.13$) and per Mcal of GEI tended ($P = 0.11$) to be reduced for the biochar treatments compared to the control whereas CH_4 per Mcal of DEI was reduced ($P = 0.07$) for the biochar cattle.

CO_2 production (g/d) was affected by treatment with 0% biochar having the greatest CO_2 production (5549 g/d) and 0.8% biochar reducing CO_2 production the most, resulting in a quadratic decrease ($P = 0.05$). This trend continued for CO_2 per kg of DMI with 0.8% biochar reducing CO_2 the most creating a quadratic response ($P = 0.06$). CO_2 production was also reduced ($P \leq 0.03$; g/d and g/kg of DMI) with the inclusion of biochar when analyzed as two treatments, with or without biochar. Adding biochar to the diet likely displaces fermentable substrate, which could result in lower CO_2 production. Leng et al. (2012a) reported greater CO_2 production from the biochar treatment relative to the control, which differs from the present trial, but did not suggest why this may have occurred. These same authors reported a lower CO_2:CH_4 ratio for the biochar-fed cattle; however, in the present study the ratio was not affected by treatment ($P \geq 0.67$). McFarlane et al. (2017) reported an increase in total gas production from an in vitro system when biochar was added to an orchard grass hay diet, but no differences were measured in volatile fatty acid concentration or ratio of acetate:propionate.

The reduction in CH_4 production reported by Leng et al. (2012a) and Saleem et al. (2018) was not observed to the same extent in the present study. Those authors reported a 24% to 25% reduction in CH_4 when feeding biochar at 0.5% to 0.6% of the diet. In the current trial, with all three treatments analyzed, CH_4 production was not statistically reduced. However, CH_4 reported as g/d and g/kg DMI tended ($P \leq 0.13$) to be reduced by biochar inclusion, 9.1% and 8.4%, respectively, when analyzed as two treatments, with and without biochar in the diet. Leng et al. (2012a) observed a 13% increase in CO_2 (ppm) when including biochar in the diet. CO_2 production was reduced approximately 8% in the current trial.

There could be many reasons for the different magnitude of results observed between the present trial and results reported by Leng et al. (2012a), including cattle breed, cattle size, diet consumed, and collection method. These authors reported that the 12 "Yellow" cattle they used had an initial BW of 80 to 100 kg, whereas in the present trial the cattle used were roughly five times that size. Rumen function and microbial population within the rumen certainly vary between cattle that are of different breed and size with differing diets and intakes, which could influence the results reported. Specific genera of bacteria and archaea have been shown to be correlated with CH_4 production, although how these microbial populations are modulated within the rumen is quite complex (Cunha et al., 2017). Leng et al. (2012a) fed a diet consisting of 61% cassava root chips and 36% cassava foliage. Cassava root is high in soluble carbohydrates and low in fiber (Oguntimein, 1988). Diet composition and quality can greatly impact CH_4 emissions, with estimates of 3.5% of GEI lost as CH_4 for concentrate-fed cattle and 6% of GEI for forage-fed cattle (Beauchemin and McGinn, 2006). In the Leng et al. (2012a) study authors used a short-term collection method for measuring respired air (once for 5 min in a headbox) and calculated CH_4 production

as described by Madsen et al. (2010). Intake drives CH_4 production, so short-term measurements are variable depending on time of gas collection relative to feeding.

The silage-based diet fed by Saleem et al. (2018) was similar to the diet fed in the current trial, but NDF and ADF content were lower. Using an artificial rumen system allows for greater control over intake, pH, passage rate, and other digestion parameters than measuring digestion in vivo, but does not perfectly replicate the animal. Results of our in vivo study matchup well with Saleem et al. (2018) in vitro study, although the magnitude of differences between treatments differ.

Finishing Experiment

Digestibility and energy. Intake of DM, OM, NDF, and ADF all increased in a bell-shaped curve ($P \leq 0.10$) as biochar inclusion in the diet increased (Table 5). DMD and OMD tended to decrease linearly ($P \leq 0.14$) as biochar inclusion increased, whereas acid detergent fiber digestibility decreased linearly ($P \leq 0.10$) as biochar inclusion increased. A linear increase ($P \leq 0.07$) in fecal ADF and fecal NDF was observed as biochar inclusion increased.

As biochar inclusion in the diet increased, GEI quadratically increased ($P = 0.07$), with 0.8% biochar having the greatest GEI (59.2 Mcal/d). Fecal energy (Mcal/d) linearly increased ($P = 0.09$) and DEI (Mcal/kg DMI) linearly decreased ($P = 0.10$) as biochar inclusion increased. There are limited data available on the impacts of biochar inclusion in finishing or high concentrate diets. Most previous research has focused on forage-based diets (Hansen et al., 2012; Leng et al. 2012a; Saleem et al. 2018). Erickson et al. (2011) fed 0, 20, or 40 g/d of an acid-washed activated carbon product made from lignite coal to dairy cows on a corn silage-based diet in two experiments. When poor quality corn silage was fed, the addition of activated carbon increased DMI and NDFD. However, when good quality corn silage was fed, no differences were measured with the inclusion of biochar. The activated carbon product fed by Erickson et al. (2011) may have had different physical and chemical properties than the biochar fed in the current study.

CH_4 and CO_2 production. Reported DMI used for gas emission calculations increased quadratically ($P = 0.01$; Table 6) as biochar inclusion increased. When biochar treatments (0.8% and 3%) were combined, biochar cattle had greater DMI ($P = 0.04$) compared to the control. Both GEI and DEI (Mcal/d) based on the 5-d headbox DMI increased quadratically ($P \leq 0.01$) as biochar inclusion increased. GEI was greater for biochar-fed cattle ($P = 0.02$) compared to the control.

Table 5. Effects of biochar inclusion in cattle diets on intake and total tract digestibility (finishing experiment)

	Biochar inclusion, % DM				*P*-value[1]	
Item	0	0.8	3	SEM	Lin	Quad
DM						
Intake, kg/d	12.0	12.9	12.1	0.51	0.84	0.07
Excreted, kg/d	3.40	3.90	3.82	0.19	0.18	0.08
Digestibility, %	71.5	70.0	68.2	1.54	0.14	0.74
OM						
Intake, kg/d	10.2	11.1	10.4	0.43	0.81	0.06
Excreted, kg/d	2.78	3.30	3.20	0.18	0.18	0.07
Digestibility, %	72.8	70.4	68.7	1.65	0.13	0.52
NDF						
Intake, kg/d	3.02	3.35	3.38	0.14	0.05	0.09
Excreted, kg/d	1.30	1.55	1.56	0.10	0.07	0.08
Digestibility, %	56.6	54.2	53.4	3.37	0.39	0.59
ADF						
Intake, kg/d	1.28	1.45	1.53	0.06	0.01	0.10
Excreted, kg/d	0.61	0.73	0.89	0.04	<0.01	0.18
Digestibility, %	52.4	50.1	41.3	3.05	<0.01	0.77
Energy						
GEI, Mcal/d	54.5	59.2	55.7	2.35	0.97	0.07
Fecal energy, Mcal/d	15.2	17.6	17.9	0.97	0.09	0.28
DEI, Mcal/d	39.3	41.6	37.8	2.12	0.35	0.25
DEI, Mcal/kg DMI	3.29	3.22	3.10	0.08	0.10	0.87

[1]Linear and quadratic orthogonal polynomial contrasts.

CH_4 production (g/d and g/kg DMI) was not different between treatments ($P \geq 0.22$) when analyzed as three treatments or as biochar inclusion vs. no biochar inclusion (Table 6). However, CH_4 production (g/d) numerically decreased 9.6% and CH_4 production (g/kg DMI) numerically decreased 18.4% for the 0.8% biochar treatment relative to no biochar. There were no differences because of treatment in CH_4 production relative to GEI or DEI ($P \geq 0.20$).

CO_2 production (g/d and g/kg DMI) was not different between treatments ($P \geq 0.34$) when analyzed as three treatments or as biochar inclusion vs. no biochar inclusion. CO_2 production (g/kg DMI) was numerically reduced 9.9% for the 0.8% biochar treatment compared to the control. The ratio of CH_4 to CO_2 was not affected by treatment ($P \geq 0.39$). Only 3 periods of data were collected in the finishing experiment (6 periods in the growing experiment) because of cattle becoming too large for the headboxes, which limited statistical power.

Table 6. Effects of increasing inclusion of biochar in cattle diets on CH_4 and CO_2 emissions from steers (finishing experiment)

	Biochar inclusion, % DM				*P*-values[1]		
Item	0	0.8	3	SEM	Lin	Quad	Y/N
DMI, kg/d	11.3	12.7	11.9	0.50	0.52	0.01	0.04
GEI, Mcal/d	51.2	58.4	54.9	2.28	0.36	0.01	0.02
DEI, Mcal/d	37.0	41.0	37.3	1.57	0.52	0.01	0.20
CH_4							
g/d	141	128	122	13.9	0.39	0.62	0.32
g/kg DMI	12.5	10.2	10.6	1.46	0.51	0.32	0.22
g/Mcal GEI	2.74	2.21	2.31	0.32	0.47	0.30	0.20
g/Mcal DEI	3.80	3.15	3.41	0.46	0.71	0.35	0.33
CO_2							
g/d	8204	8402	7755	558	0.50	0.66	0.86
g/kg DMI	737	664	664	61.4	0.52	0.51	0.34
CH_4:CO_2	0.017	0.016	0.016	0.0019	0.56	0.56	0.39

[1]Linear and quadratic orthogonal polynomial contrasts. Y/N = biochar inclusion in diet (0.8% and 3% treatments combined) vs. no biochar in diet (0 treatment).

The effect of biochar on CH_4 production from ruminants has not been explored in depth, but has shown promise as a potential mitigation strategy. Hansen et al. (2012) and Leng et al. (2012b) both reported 10% to 17% reductions in CH_4 emissions from in vitro systems when biochar was included, although Hansen et al. (2012) did not report statistically significant differences. Saleem et al. (2018) reported a linear increase in digestibility of DM, OM, ADF, and NDF with a 25% reduction in CH_4 production when adding 0.5% engineered biocarbon to an artificial rumen system. Biochar used in the Hansen et al. (2012) and Saleem et al. (2018) studies was made from wood or straw whereas biochar was derived from rice husks in the Leng et al. (2012a, 2012b, 2013) studies. In vitro runs are variable and do not replicate what happens inside the animal perfectly as there are sources of error involved in the procedure. For this reason, the in vivo experiments were conducted. Although not always statistically significant, there were consistent numerical decreases in CH_4 production with 0.8% biochar inclusion in the diet compared to no biochar. Intake was not hindered with biochar inclusion, and actually increased in the finishing experiment. Feeding 0.8% biochar appears to be sufficient and no further benefits were observed from increasing inclusion to 3% of diet DM. The effects of biochar in the rumen show promise, but are not fully understood and performance data (BW gain, efficiency, and carcass data) are needed to determine if it is a feasible CH_4 mitigation tool for beef cattle.

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