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Effect of Biochar Supplementation on Grazing Beef Cow and Calf Performance, Enteric Methane and Carbon Dioxide Emissions, Fecal Egg Counts and Fecal Nutrient Composition

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Abstract

A three-year beef cow grazing study (2020-2022) was conducted to evaluate the effects of biochar supplementation on grazing cow performance, ruminal fermentation, fecal egg and oocyst count, fecal nutrient composition, and enteric methane (CH_4) and carbon dioxide (CO_2) emissions. Each year, forty-eight spring-calving Angus beef cows (BW = 669 ± 95 kg; mean ± STD) stratified by BW, were randomly assigned to 1 of 2 treatments. Treatments included either (i) pelleted supplement with biochar (Biochar; 405 g/d**)** inclusion at 2.2% of DMI or (ii) control pellet (Control; base fiber - no biochar). Twenty-four ha meadow bromegrass (*Bromus riparius* Rehm.)-alfalfa (*Medicago sativa* L.) paddocks (8.6% CP, 51.1% TDN) were assigned to each treatment group for the 92-d grazing trial. Enteric CH_4 and CO_2 emissions were measured using SF_6 tracer gas technique. Cow and calf performance, rumen fluid parameters were not ($p > 0.05$) affected by biochar supplementation. During trial biochar supplementation reduced fecal oocyst (*Eimeria spp*.) count ($p < 0.011$; 3.9 vs. 15.0 count/g DM) but increased carbon:nitrogen ratio ($p < 0.029$; 26.1 vs. 21.3) relative to initial measurements. The enteric methane emission reduction in response to biochar supplementation at 2.2% of DMI, was negligible (~6.0% reduced compared to control).

Keywords: biochar, carbon dioxide, cattle performance, intestinal parasites, manure, methane emission

1. Introduction

Improving forage utilization is critical for the profitability of all sectors of the beef cattle industry. Methane (CH_4) and carbon dioxide (CO_2) are natural byproducts of ruminant fermentation, with total gas production being primarily dependent on the microbial efficiency of feed conversion (Hansen, Storm, & Sell, 2012). Methane has a global warming potential that is 28 times higher than CO_2 on a 100-year horizon (Myhre et al., 2013). Hence, the cattle industry has an increasing incentive to develop strategies to mitigate enteric CH_4 production. Dietary manipulation using novel additives may serve to achieve this objective. Biochar is generated as a result of the partial pyrolysis of organic matter and although primarily used as a soil amendment (Schmidt, Hagemann, Draper, & Kammann, 2019), it has recently been reported to enhance feed degradability and possibly lower enteric CH_4 emissions (Leng et al., 2012a,b; 2013). Biochar pyrolyzed at high temperature in a manner that generates a very high surface area is called engineered or activated biochar and has been theorized to promote the formation of microbial biofilms in the rumen (Leng, Inthapanya, & Preston, 2012a; Leng, 2014), a process essential for ruminal feed digestion (McAllister, Bae, Jones, & Cheng, 1994). Further, biochar may lower the production of ruminal CH_4 emissions both in vitro (Hansen et al., 2012; Leng et al., 2012a) and in vivo (Leng, Preston, & Inthapanya, 2012c). It has been suggested that biochar reduces ruminal enteric CH_4 emissions by altering rumen microbial biofilms, decreasing rumen methanogens and increasing rumen methanotrophs (Leng et al., 2012a,b,c; Toth & Dou, 2016). Addition of biochar to a forage diet increased DM digestibility by up to 2%, while lowering enteric CH_4 emissions and enhancing microbial protein synthesis in vitro semi-continuous culture fermenters (Saleem et al., 2018). Previous studies also found evidence of reduction intestinal parasites when

charcoal was fed to domestic animals (Van, Mui, & Ledin, 2006; Watarai & Koiwa, 2008; Paraud et al., 2011). However, field studies involving supplementing biochar to grazing beef cows are limiting. This experiment aimed to evaluate biochar supplementations effect on cow and calf performance and health, enteric greenhouse gas (GHG) emissions, and rumen fermentation in grazing beef cattle.

2. Materials and Methods

2.1 Experimental Site, Trial Design, and Grazing Management

A three-year (2020-2022) beef cow grazing study with two treatments was conducted at the University of Saskatchewan's Livestock and Forage Centre of Excellence - Forage and Cow-Calf Unit located at Clavet, Saskatchewan, Canada. Soil at Clavet site is classified as an Orthic Dark Brown Chernozem, Shellbrook-Hamlin association on a nearly level topography of very fine sandy loam to loam texture (Saskatchewan Soil Survey, 1999). A forty-eight-ha meadow bromegrass (*Bromus riparius* Rehm)-alfalfa (*Medicago sativa* L.) pasture was subdivided into two twenty-four-ha paddocks. Each paddock was randomly assigned to 1 of 2 treatments: either (i) Biochar (biochar-based pellet) or (ii) Control (base fiber - no biochar in pellet). Both pellets were produced by the Canadian Feed Research Centre, North Battleford, Saskatchewan, Canada. Pine-sourced biochar was purchased from Oregon Biochar Solution (White, OR, USA). The particle size distribution of the biochar was 1.4%, 3.2%, 49.8%, and 43.9% for 0.5 mm, 0.5-1 mm, 1-2 mm, 2-4 mm, and 4-8 mm, respectively. The biochar was incorporated into a pellet (thereafter, biochar pellet; 12.6% CP, 59.7% TDN, 1.13% Ca, 0.61% P) containing 45% biochar, 45% wheat midds, and 10% canola. Control pellet was (14.3% CP, 77.5% TDN; 3.8% Ca; 0.62% P) and consisted of 90.0% wheat midds and 10.0% canola oil. Cow/calf pairs were provided treatment pellets (Biochar or Control), at a rate of 500 g/d (control; 460 g/d DM basis) per cow or 1000 g (biochar; 900 g/d DM basis) in a portable feed trough once daily in the morning (~0800 h). Biochar dose was fed at ~2.2% (or 0.058% of BW) of estimated DMI.

Grazing trials were conducted for 89 d (21 d adaptation and 68 d data collection, 31 July to - 7 October 2020) in yr 1; for 89 d (21 d adaptation and 68 d data collection, 29 June to - 2 September 2021) in yr 2, and 97 d (21 d adaptation and 76 d data collection, 24 June to - 8 September 2022) in yr 3. Before the 2020 trial start and based on initial cow BW, we estimated that to be within 10 units of mean BW with 80% power at the 5% significance level (Kuehl, 2000), it would require a minimum of 24-25 animals per treatment group. Therefore, each year, forty-eight spring-calving Angus beef cows (BW = 669 ± 95 kg; mean ± STD) were stratified by BW and randomly assigned to 1 of 2 treatments.

2.2 Animal and Pasture Measurements

The trial was pre-approved by the University Animal Care Committee of the University of Saskatchewan (Protocol No. 20090107) and all animals were managed according to the (Canadian Council of Animal Care (2009). Cow BW, subcutaneous fat (rib fat, mm) thickness and *longissimius dorsi* fat (rump fat, mm) reserves were used as indicators of animal performance and were taken at the start (initial BW) and end (final BW) of the grazing trial, on 2 consecutive days in the morning. The calf BW was taken at the start (initial BW) and end (weaning BW) of the trial. All cow BW data were adjusted for conceptus weight and associated fluids according to the equation (NASEM, 2016).

$$Conceptus\ weight\ (kg) = (calf\ birth\ weight \times 0.01828) \times e^{[((0.02 \times t) - (0.0000143 \times t \times t)]} \quad (1)$$

Where, t = d of pregnancy. Date of conception was determined by subtracting 285 d from subsequent calving date (DeRouen et al., 1994).

Calves were weaned on 6 and 8 October for yr 1 and yr 2, respectively. The pre-weaning ADG for calves was calculated for each animal by subtracting the initial weight from the weaning weight and dividing by the number of days on the trial. Rib and rump fat thickness of the live animal were measured with ultrasound as described by Bergen, McKinnon, Christensen, Kohle, & Belanger (1997) using an Aloka SSD-500V real-time ultra-sound machine and Aloka UST-5044 probe (3.5 MHz, Aloka Inc., Wallingford, CT).

Forage yield (FY; kg DM/ha) of pre- and post-grazing (within 1-2 days before and after grazing trial) was determined by randomly clipping 40, 0.25-m^2 quadrats in each paddock to a stubble height of 2 cm. Forage utilization (FU) and weight of dried available and residual forages were estimated according to Cook and Stubbendieck (1986) equation:

$$FU\ (\%) = (pre\text{- }grazing\ FY,\ DM\ (g/m^2) - post\text{-}grazing\ FY\ (g/m^2))/(pre\ -\ grazing\ FY\ (g/m^2)) \times 100 \quad (2)$$

The DMI was estimated using Minson and McDonald (1987) equation:

$$DMI = (1.185 + 0.00454midBW - 0.0000026BW^2 + 0.315ADG)^2 \quad (3)$$

where DMI = Dry matter intake (kg/d) for cattle on pasture using animal performance; MBW, mean body weight (BW, kg) is average BW (kg) for the experimental period [(initial BW + final BW)/2]; ADG, average daily gain (kg/d) for individual cows.

All animals had *ad libitum* access to a commercial 2:1 mineral supplement (11.5% Ca, 10% P, 1% Mg, 5.8% Na, 200 ppm I, 4900 ppm Fe, 2000 ppm Cu, 5000 ppm Mn, 5000 ppm Zn, 20 ppm Co, 50 ppm Fl, 500000 IU/kg vitamin A (min), 50000 IU/kg vitamin D_3 (min), 2500 IU/kg vitamin E (min) (PerforMax®, Blairs, Lanigan, Saskatchewan, Canada) and a cobalt iodised salt block. Cows with calves were group managed.

2.3 Measuring Enteric CH_4 and CO_2 Emissions

Each year and for each treatment, five cows were randomly selected for enteric GHG (CH_4 and CO_2) emissions measurement. Enteric GHG emissions were measured using SF_6 tracer gas technique (CH_4) according to Johnson and Johnson (1994). The principles of this technique are based on a known SF_6 gas release rate from a permeation tube, dosed into the rumen, and continuous subsample collection of exhaled breath into an evacuated canister. The CH_4 emissions can be estimated from the SF_6:CH_4 ratio in the canister and the SF_6 permeation rate, with corrections for background air values of SF_6 and CH_4 (Lassey, Pinares-Patiño, Vlaming, Smith, & Clark, 2011). Brass permeation tubes containing the SF_6 tracer were prepared by Agriculture and Agri-Food Canada and kept at 39°C as described by Lassey et al. (2011) and the permeation rate was determined by weighing the tubes at weekly intervals over 4 months (for linear regression; $r^2 > 0.999$), before dosing the animals 3 wks before the first breath collection measurements. The average SF_6 permeation rate from the permeation tubes used over 3-yr period was 5.98 ± 1.36 mg/d. Brass permeation tubes containing SF_6 with a predetermined release rate were placed by bolus gun in the rumen of each cow 14 d prior to the experiment start to al-low for tracer gas to reach a steady state (Iwaasa, Stumborg, Wittenberg, McGinn, & McAllister, 2004).

The SF_6 tracer gas sampling was conducted once daily over 4-d period, during 2 periods. Therefore, animals were fitted with halters and gas collection canisters for 5 d following the 4-d acclimation to the treatment in each of two periods for CH_4 and CO_2 measurements. Every 24 h exhaled gases from animals' collection yokes were sampled using procedure described by Chaves et al. (2006) and Iwaasa et al. (2004). Background air samples were collected during the sampling period by hanging 4 gas collection equipment (yoke, halter) in each corner of the study pasture and were replaced daily. Gas (breath and background air samples) were collected with a 10-cc syringe and placed in a pre-evacuated excutainer and stored at room temperature, then CH_4 and CO_2 concentrations were determined via gas chromatography (Agilent 7890B series GC custom, Agilent Technologies Canada Inc., Mississauga, ON, CA). All the samples were analyzed in triplicate. Criteria for ensuring sample integrity are described in Lassey et al. (2011) to identify (and reject) samples associated with leaks and blockages or low concentrations of SF_6 and CH_4 samples relative to background air. Around 10% of all samplings were excluded from analysis, mainly due to blockage or leaking of the sampling line or extremely low SF_6 gas concentrations relative to the background.

Enteric CH_4 emissions was calculated on an individual cow basis by calculating the average CH_4 for each day in the 4-day sampling week, and then taking the average of all 4 days to calculate a weekly (or period) CH_4 value.

The CH_4 daily emission was calculated as follows:

$$CH_4\,(g/d) = SF_6\ permeation\ rate \times [(CH_4\ sample - CH_4\ background)/(SF_6\ sample - SF_6\ background)] \times (16/146) \times 1000 \quad (4)$$

Where SF_6 permeation rate is expressed in milligrams per day; CH_4 sample and CH_4 background are expressed in micromoles per mole; SF_6 sample and SF_6 background are expressed in picomoles per mole; 16 and 146 are the molecular weight (g/mol) of CH_4 and SF_6, respectively; and the multiplier 1000 accounts for the different units for CH_4 and SF_6 in the equation. Enteric CO_2 emissions was calculated similar manner with CH_4. Simultaneously, the CH_4 and CO_2 emission yield was calculated as daily emission per DMI (g/kg DMI). The ratio of CH_4 to CO_2 (CH_4:CO_2) and quantity of GHG was expressed as CO_2-eq was calculated according to Brander & Davis (2012).

2.4 Forage Sampling for Quality Analysis

To estimate forage nutritive value, in each paddock, 10 randomly distributed quadrats (0.25 m^2) were clipped and composited in plastic sample bags. Two subsamples from each composite sample at the start, middle, and end of trial were placed in paper bags and dried in a forced-air oven at 55°C for 72 h, then ground to pass through a 1-mm screen using a Wiley mill (Model 4, Arthur H. Tomas Co., Philadelphia, PA) and stored in a refrigerated room (4°C) until further analysis. Samples were analyzed for dry matter (DM; AOAC method# 930.15), ash (AOAC method# 942.05), crude fat (AOAC method# 920.02), and crude protein (CP; AOAC method# 984.13)

contents according to the procedure of AOAC (2000). Crude protein was determined using a Leco FP-2000 nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA) and acid detergent fiber (ADF) and neutral detergent fiber (NDF) with heat stable α-amylase were determined using an ANKOM Fiber Analyzer (ANKOM Technology Corporation., Fairport, NY, USA). Forage TDN were determined according to Weiss, Conrad, & Pierre (1992).

2.5 Rumen Fermentation Profile

Each year, twenty-four cows (12 cows for each of two treatments) were randomly selected for rumen fluid sampling. At the end of trial, the rumen fluid was collected by inducing flexible plastic tubing into the rumen and using a suction pump to secure a sample with the first 100 ml of fluid being discarded to avoid saliva contamination, and then ~100-150 ml of rumen fluid was strained with a double layer of cheese cloth (to remove coarse particles) into a plastic beaker (~250 ml). From the filtrate, 8 ml duplicates were collected (using a syringe) and transferred to the marked test tubes for short-chain fatty acids (SCFA) and NH_3-N analysis. The test tubes for SCFA and NH_3-N contained 2 ml each of metaphosphoric acid (HPO_3) and sulphuric acid (H_2SO_4), respectively, as preservatives. The contents were then mixed after adding rumen fluid before storage at -20°C until analysis. The SCFA concentrations were determined by gas chromatography (Agilent 6890; Agilent Technologies, Inc., Mississauga, ON, Canada) using the procedure described by Khorasani, Okine, & Kennelly (1996). Measurement of ruminal NH_3-N concentration was completed using the phenol-hypochlorite method as described by Broderick and Kang (1980).

2.6 Fecal Sampling and Analysis

Each year, fecal rectal grab samples were obtained from 12 animals per treatment at 2 time points at the start (before trial) and end of the trial, and fecal worm egg counts and nutrient composition were determined. Fecal egg and oocyst counted using Modified Wisconsin Sugar Flotation Technique (Bliss & Kvasnicka, 1997). These methods have been developed to recover parasitic worm eggs and oocysts (from protozoan parasites), as they pass out of the animals, to identify them according to the type of parasite present and to enumerate them as to the number of worm eggs being passed in a specific amount of fecal material. Fecal rectal grab samples were also subsampled and analyzed for DM, total C, total N, total K, available P, and C:N ratio. Chemical composition analysis of the fecal samples was conducted by A&L Canada Laboratories Inc. (London, ON, Canada). The total N and total P were determined according to Thomas, Sheard, & Moyer (1967). Total K was determined using inductively coupled plasma emission spectroscopy Perkin Elmer Optima 3000DV (Norwalk, CT).

2.7 Weather

Average monthly temperature and precipitation data were obtained from the Environment Canada's Climate Data website (http://www.comiate.weatheroffice.ec.gc.ca) for Saskatoon, Saskatchewan (Climate ID 4057165; 52°17'N, 106°72'W), Canada. Three-year average monthly total precipitation for the study site for June, July, August, September, and October were 62.2, 38.8, 26.7, 12.0, and 5.1 mm, respectively. The 30-yr average (LTA) monthly precipitation at the study site for June, July, August, September, and October were 63.6, 53.8, 44.4, 38.1, and 18.8 mm, respectively (Table 1). The 3-yr average monthly temperatures at the study site for June, July, August, September, and October were 16.3, 19.9, 18.5, 13.5 and 4.6°C, respectively. The LTA monthly temperatures at the study site for June, July, August, September, and October were 16.1, 19.0, 18.2, 12.0, and 4.4°C, respectively. Thus, the current study trials were conducted in an environment with similar temperatures and 25% lower precipitation compared to the LTA.

Table 1. Monthly average air temperature and precipitation in Saskatoon, Saskatchewan, Canada during the trials over 3 yr (2020 to 2022)[1]

	Year				
Month	2020	2021	2022	3-yr avg.	LTA[2]
Temperature, °C					
June	15.3	18	15.7	16.3	16.1
July	19	21.4	19.3	19.9	19
August	18	17.8	19.6	18.5	18.2
September	11.7	13.7	15.0	13.5	12
October	1.24	5.5	7.1	4.6	4.4
Precipitation, mm					
June	106.9	41.7	38	62.2	63.6
July	52.1	17.7	46.5	38.8	53.8
August	16.2	38.4	25.6	26.7	44.4
September	23.6	5.6	6.8	12.0	38.1
October	3.5	6.7	5.1	5.1	18.8

Note. [1]Data were obtained from Environment Canada (www.climate.weatheroffice.ec.gc.ca) for Saskatoon (Climate ID 4057165; 52°17'N, 106°72'W).

[2]LTA, Long-term average from 1981 to 2010.

2.8 Statistical Analysis

Animal performance data including BW, ADG, rib fat, rump fat, BCS, CH_4 and CO_2 emissions, CH_4:CO_2, rumen fermentation profile, calf BW, and calf pre-weaning bodyweight were analyzed using the MIXED procedure of SAS 9.2 (2003) as a completely randomized design. The model used for the analysis was: $Y_{ij} = \mu + T_i + e_{ij}$; where Y_{ij} was an observation of the dependent variable $_{ij}$; μ was the population mean for the variable; T_i was the fixed effect of the supplementation system [(Biochar; or (ii) control (Control-base fiber - no biochar supplementation)]; and e_{ij} was the random error associated with the observation $_{ij}$.

Fecal egg and oocyst counts and fecal nutrient composition were analyzed and subjected to a two-way analysis of variance (year and treatment) with SAS 9.2 (2003): the whole-plot experimental unit was treatment (four treatments included: CON-Initial; CON-Final; Biochar-Initial; and Biochar-Final) and the subplot experimental unit was period (initial and final) within the treatment. The model used was: $Y_{ijr} = \alpha_i + \beta_j + e_{ijr}$; where Y_{ijr} is the variable studied, αi is the treatment, β_j is the year effect, and eijr is the residual standard deviation used as the error term. For the enteric CH_4 and CO_2 emissions, data were analyzed as repeated measures according to a completely randomized design using the MIXED procedure of SAS (2003). The MIXED model included treatment as fixed effects and random effects of cow, with sampling period as the repeated measure. Each cow was considered an experimental unit. Year was treated as a random variable in all analyses and differences between treatment means were determined using Tukey's multiple range test and were considered significant when $p < 0.05$ and trends were discussed when $p < 0.10$.

3. Results

3.1 Forage Yield, Utilization, and Nutrient Composition

The effects of biochar on forage utilization and nutrient composition over the 3-yr study are presented in Table 2. Forage pre-grazing yield averaged 2811 ± 590 kg/ha and was not different ($p = 0.856$) between paddocks, indicating even forage distribution throughout the pasture. Likewise, forage utilization due to grazing was similar between paddocks ($p = 0.153$) averaging 57.3 ± 17.5% of available forage. Also, forage CP (8.6 ± 0.40% of DM), ADF (44.1 ± 7.7% of DM), NDF (64.5 ± 7.7% of DM), TDN (51.1 ± 9.9% of DM), Ca (0.6 ± 0.3% of DM), and P (0.10 ± 0.06 % of DM) contents did not vary ($p > 0.05$) between treatments.

Table 2. Forage yield, forage utilization, and nutrient composition of pasture forage over 3 yr (DM basis)

	Treatment[1]			
Item	Control	Biochar	SEM	*p*-value
Forage yield, kg DM/ha				
Pre-grazing	2759.2	2863.7	381.03	0.856
Post-grazing	926.3	1505.4	263.90	0.196
Forage utilization				
kg/ha	1833.0	1358.3	325.35	0.361
%	67.8	46.7	8.48	0.153
Nutrient composition, % DM				
CP	8.4	8.8	0.06	0.585
ADF	44.6	43.6	1.17	0.526
NDF	65.4	63.6	1.53	0.415
TDN[2]	50.7	51.5	1.50	0.704
Ca	0.55	0.64	0.043	0.0145
P	0.13	0.12	0.009	0.491

Note. [1]Control = paddock assigned for no biochar supplemented; Biochar = paddock assigned for biochar supplemented at 2.2% (405 g/d) of estimated DMI.

[2]TDN were determined according to Weiss et al. (1992).

3.2 Effect of Biochar Supplementation on Cow and Calf Performance

Animal performance and estimated DMI are presented in Table 3. Cow initial BW (677 ± 92 kg; mean ± STD) and final BW (671 ± 79.0 kg) did not change ($p > 0.05$) among the cow groups. No differences ($p > 0.05$) were observed between the cow groups on rump fat thickness (5.1 ± 1.7 mm).

Table 3. Effect of biochar supplementation on grazing beef cow and calf performance over 3 yrs

	Treatment[1]			
Item	Control	Biochar	SEM	*p*-value
n, animals	72	72	-	-
Cow performance				
Initial BW, kg	679	659.3	11.33	0.218
Final BW, kg	684.9	661.3	9.43	0.078
BW change, kg	5.9	2.1	4.39	0.536
Initial rib fat, mm	4.44	4.39	0.148	0.78
Final rib fat, mm	4.26	4.26	0.121	0.969
Rib fat change, mm	-0.18	-0.13	0.188	0.845
Initial rump fat, mm	5	5.28	0.252	0.445
Final rump fat, mm	5.08	5.58	0.306	0.255
Rump fat change, mm	0.38	0.67	0.223	0.36
DMI[2], kg/d	9.65	9.2	0.107	0.003
DMI[2], % BW	1.45	1.45	0.023	0.894
Calf weaning performance[3]				
Calf initial BW, kg	154.6	145.3	6.01	0.276
Calf weaning BW, kg	237.5	238.7	5.74	0.885
Calf BW change, kg	82.9	93.8	5.43	0.161
Pre-weaning ADG, kg/d	0.98	1.13	0.069	0.119

Note. [1]Control = No biochar supplemented; Biochar = Supplemented with biochar at 2.2% (405 g/d) of estimated DMI.

[2]DMI was estimated on an individual basis using cow BW and ADG according to Minson and McDonald (1987).

[3]Days of calculation of calf ADG were 68 and 101 for yr 1 and yr 2 (2020 and 2021), respectively.

There was a trend for the cows supplemented with biochar to have lower final BW ($p = 0.078$). Cows supplemented with biochar had lower ($p = 0.003$) DMI relative to control group (9.14 ± 0.95 vs 8.94 ± 1.98 kg/d).

However, when DMI expressed in percentage of BW, estimated forage intake (DMI) averaged 1.45 ± 0.20% of BW and was not different (p = 0.894) between cow treatment groups.

Calf initial BW (150.0 ± 41.0 kg; mean ± STD), weaning (final) BW (238.1 ± 39.2 kg), and ADG (1.05 ± 0.47 kg/d) did not vary between the treatments. Thus, the results of this study indicated that measures of animal performance, including BW, and subcutaneous fat (rib fat, mm) thickness, and *longissimius dorsi* fat (rump fat, mm) were not affected (neither negatively nor positively) by biochar supplementation.

3.3 Effect of Biochar Supplementation on Enteric GHG Emissions

Effect of biochar supplementation on daily enteric gas (CH_4 and CO_2) emissions and yield in grazing beef cows is shown in Table 4.

Table 4. Effect of biochar supplementation on enteric gas (CH_4 and CO_2) daily emissions and yield in grazing beef cows over 3 yrs

	Treatment[1]			
Item	Control	Biochar	SEM	*p*-value
n, animals	15	15	-	-
Daily emission, g/d				
CH_4	314.1	298.8	17.07	0.538
CO_2	10613.3	10468.1	632.77	0.873
Emission yield, g/kg DMI				
CH_4	32.7	30.8	1.88	0.478
CO_2	1108.9	1072.7	74.42	0.736
CO_2-eq kg/d	7.85	7.23	0.45	0.347
CH_4:CO_2, mol/mol	0.08	0.08	0.004	0.564

Note. [1]Control = No biochar supplemented; Biochar = Supplemented with biochar at 2.2% (405 g/d) of estimated DMI.

No difference was observed (p = 0.538) between the two treatments in enteric CH_4 daily emissions (308.7 ± 46.7 g/d). Likewise, enteric CO_2 emission was not affected (p = 0.873) by biochar supplementation and averaged 10561 ± 1708 g/d. Consequently, the molar CH_4:CO_2 ratios were similar (p = 0.564) among the cow groups (0.085 ± 0.016). The CH_4 emission expressed as kg CO_2-eq/d was also similar (p = 0.347) among the cow groups (7.66 ± 1.18 CO_2-eq kg/d). Enteric emissions yield of CH_4 (32.14 ± 4.86 g/kg DMI) and CO_2 (1097.6 ± 189.3 g/kg DMI) did not differ ($p > 0.05$) between the cow groups with different supplementation.

3.4 Effect of Biochar Supplementation on Ruminal SCFA and Ammonia Nitrogen

There was no effect ($p > 0.05$) of biochar supplementation on rumen fluid total SCFA concentration (63.9 ± 11.2 mmol/L; mean ± STD), acetate:propionate ratio (5.0 ± 0.5) or rumen ammonia-N concentration (7.0 ± 4.9 mg/dL; Table 5). Likewise, the molar proportions of acetate (75.8 ± 3.0%), propionate (15.1 ± 2.2%), butyrate (7.8 ± 1.3%), valerate (0.2 ± 0.4%), and isobutyrate (0.9 ± 0.9%) were similar ($p > 0.05$) among the cow groups.

Table 5. Effects of biochar supplementation on ruminal short-chain fatty acid (SCFA) concentration and rumen ammonia nitrogen (NH_3-N) of grazing beef cows over 3 yrs (2020 - 2022)

	Treatment[1]			
Item	Control	Biochar	SEM	*p*-value
n, animals	36	36	-	-
Acetate, % total SCFA	75.7	75.8	0.5	0.948
Propionate, % total SCFA	15.3	15	0.37	0.564
Butyrate, % total SCFA	7.7	7.8	0.22	0.989
Valerate, % total SCFA	0.2	0.3	0.06	0.469
Isobutyrate, % total SCFA	0.8	1	0.17	0.443
Total SCFA, mmol/L	63.9	67.7	2.06	0.201
Acetate:propionate ratio	5	4.9	0.09	0.749
Rumen NH_3-N, mg/dL	6	8.1	0.82	0.129

Note. [1]Control = No biochar supplemented; Biochar = Supplemented with biochar at 2.2% (405 g/d) of estimated DMI.

3.5 Effect of Biochar Supplementation on Fecal Egg Counts and Fecal Nutrient Composition

Fecal egg and oocyst count over 3 yr are presented in Table 6. There were 2 types of parasites detected, *Eimeria* spp. and *Trichostrongyle* species. The *Eimeria* species were in oocysts, whereas *Trichostrongyle* species were in egg stage. When initial and final fecal parameters were compared, biochar supplementation decreased ($p = 0.011$) *Eimeria sp.* oocysts level by 3.9 (14.92 vs. 3.85 count/g DM) times. In contrast, in control cows through the trial, *Eimeria spp*. oocysts counts numerically increased ($p = 0.459$) by 97% (10.3 vs. 20.30 count/g DM). No differences ($p > 0.05$) were detected on *Trichostrongyle* species among cow groups.

Effect of biochar supplementation on fecal nutrient composition (% of DM) of grazing beef cows is shown in Table 7. Treatment interactions were not observed for any measured parameters ($p > 0.05$) collected at the end of trial. Cow feces of control treatment had lower total nitrogen (2.2 vs. 1.6%; $p = 0.002$) and phosphate (1.3 vs. 1.0%; $p = 0.049$) in the final period relative to the initial period of the grazing trial. However, as expected, feces of biochar cows contained greater ($p < 0.05$) organic matter (75.1 vs. 70.9%), total carbon (41.6 vs. 39.5%), and carbon:nitrogen ratio (26.1 vs. 21.3) but contained lower potassium ($p = 0.037$) in the final relative to the initial period of the grazing trial.

Table 6. Effects of biochar supplementation on fecal egg and oocyst (count/g DM) of grazing beef cows over 3 yrs (2020-2022)

	Treatment[1]			
Item[2]	Control	Biochar	SEM	*p*-value
n, animals	36	36	-	-
Trichostrongyle sp.				
Initial	10.74	15.99	3.984	0.377
Final	3.58	10.83	2.984	0.079
SEM	3.356	3.677	-	-
p-value	0.138	0.325	-	-
Eimeria sp.				
Initial	10.29	14.92	4.068	0.446
Final	20.30	3.85	8.572	0.163
SEM	9.483	2.968	-	-
p-value	0.459	0.011	-	-

Note. [1]Control = No biochar supplemented; Biochar = Supplemented with biochar at 2.2% (405 g/d) of estimated DMI.

[2]Counted by the Wisconsin Sugar Flotation Technique (Bliss and Kvasnicka, 1997); *Eimeria* species were in oocysts and *Trichostrongyle* species were in egg stage.

Table 7. Effect of biochar supplementation on fecal nutrient composition (% DM) of grazing beef cows

	Treatment[1]			
Item	Control	Biochar	SEM	*p*-value
n, animals	36	36	-	-
Total nitrogen				
Initial	2.2	1.9	0.12	0.069
Final	1.6	1.6	0.11	0.883
SEM	0.12	0.11	-	-
p-value	0.002	0.073	-	-
Phosphate (as P_2O_5)				
Initial	1.3	1.1	0.14	0.287
Final	1.0	1.0	0.07	0.689
SEM	0.10	0.12	-	-
p-value	0.049	0.442	-	-
Total potassium				
Initial	0.96	1.23	0.10	0.076
Final	0.81	0.97	0.08	0.197
SEM	0.106	0.080	-	-
p-value	0.354	0.037	-	-
Potash (K as K_2O)				
Initial	1.1	1.5	0.12	0.076
Final	1.0	1.2	0.10	0.197
SEM	0.13	0.10	-	-
p-value	0.354	0.037	-	-
Organic matter				
Initial	70.6	70.9	1.16	0.842
Final	74.0	75.1	1.51	0.645
SEM	1.68	0.89	-	-
p-value	0.172	0.006	-	-
Total carbon				
Initial	39.4	39.5	0.63	0.875
Final	41.4	41.6	0.78	0.905
SEM	0.90	0.45	-	-
p-value	0.121	0.005	-	-
Carbon:nitrogen ratio				
Initial	17.8	21.3	1.15	0.0486
Final	27.0	26.1	1.81	0.7375
SEM	1.61	1.42	-	-
p-value	0.001	0.029	-	-

Note. [1]Control = No biochar supplemented; Biochar = Supplemented with biochar at 2.2% (405 g/d) of estimated DMI.

4. Discussion

4.1 Forage Yield, Utilization, and Nutrient Composition

The recommended minimum forage yield to maintain a desirable grazing efficiency is reported to be 2000 kg/ha (Coleman, 1992; Baron, Dick, Bjorge, & Lastiwka, 2005; Kulathunga, Penner, Schoenau, Damiran, Larson, & Lardner, 2016) suggesting that the forage yield by later stage of the current trial may limit intake. Forage utilization in grazing systems depends upon how long the livestock are held on an area to utilize remaining forage (Damiran, Lardner, Larson, & McKinnon, 2016). The utilization of the forage stand at this site was at the targeted level. Grazing animals have different nutritional requirements based on their stage of production. During the mid-gestation period, the CP requirement is lower, ranging from 6.9% to 7.1% DM (NASEM, 2016). Based on NASEM (2016), forages in both paddocks met the minimum CP requirement for beef cows with similar weight and gestation stage to the animals used in the current study. Using TDN as the energy source for beef cows, the rule of thumb is 55-60-65 (%, DM) (Yurchak & Okine, 2004). This rule suggests that for a mature beef

cow to maintain her body condition score, the ration must have a TDN energy reading of 55% during in mid pregnancy, 60% in late pregnancy and 65% post calving (Yurchak & Okine, 2004). Therefore, in the current study, forages in both paddocks (treatments), had slightly lower energy content than what is needed by nursing and gestating cows, even for early stages of pregnancy. Furthermore, according to NASEM (2016), grasses or grass-legume mixtures with greater than 600 g/kg NDF are considered of low quality. Hence, the NDF content of the pasture in the current study would suggest of a low-quality forage. It is likely that low precipitation during the summer played a major role in the lower nutritive value or quality of forages in the current study.

4.2 Effect of Biochar Supplementation on Cow and Calf Performance

Despite the lower energy content of pasture forage, then considering the cow performance data (BW, rib and rump fat), it can be implied that cows in both treatment paddocks adequately met the NASEM (2016) maintenance nutrient requirements (with only slight BW gain). As evident from Table 2, forage nutritive value was similar between the biochar and control paddocks, hence cow performance would be expected to also not differ. Also, that no evidence of either positive or negative effect, due to biochar supplementation on cow performance were observed in the present study is supported by Terry et al. (2019a, 2019b) and Winders et al. (2019) studies conducted on effect of biochar supplementation on beef cattle performance.

Trend for cows supplemented with biochar having lower final BW, which could be attributed to the numerical variation in initial BW of animals. When initial animal weight was used as a covariate, the final BW was not different ($p = 0.702$; 683 vs. 680 kg control and biochar cows, respectively; data not shown). In the current study, the DMI calculation was based on animal weight and requirements. Therefore, it is expected that DMI should be different between the cow groups. However, when initial animal weight was used as a covariate then DMI kg/d ($p = 0.175$) and DMI % of BW ($p = 0.411$) were not different among the cow groups.

Likewise, estimated DMI (expressed percentage of BW) was similar between treatments, and this was consistent with previous experiments by others (Winders et al., 2019; Terry et al., 2019a; Terry et al., 2019b), where biochar supplementation had no significant effect on steers and heifers DMI fed high-forage or high-concentrate diets. Furthermore, Daley, McCuskey, & Bailey (1987) reported a significant positive correlation ($r = 0.39$) between milk protein yield of *B. taurus* and *B. taurus* × *B. indicus* cows and pre-weaning weight of their calves. Therefore, no variation ($p > 0.500$) among treatments on calf performance in the current study suggested no differences between cow groups in milk yield and composition.

4.3 Effect of Biochar Supplementation on Enteric GHG Emissions

Enteric CH_4 is formed in the rumen primarily from hydrogen produced during ruminal fermentation, particularly with high fiber diets, with diets that shift fermentation pathways to favor propionate over acetate and butyrate, generating less CH_4 (Noviandi et al., 2014). Since there was no shift in fermentation pathways to favor propionate over acetate and butyrate observed in the current study, the reduction of CH_4 emissions due to biochar supplementation was negligible (only about ~6% compared to control) and less consistent. Currently, only a few experiments have investigated biochar supplementation to beef cows, of which none have been conducted managing beef cattle grazing on pasture in a field trial. Since the cow-calf sector has been implicated as a major contributor of enteric CH_4 emissions within the beef industry (Legesse et al., 2015), it is important to investigate opportunities for CH_4-mitigation within this area of the production cycle. Enteric CH_4 emission of grazing cattle is highly dependent on forage quality. For example, Westberg, Lamb, Johnson, & Huyler (2001), noted a large seasonal variation in enteric CH_4 emissions of beef cows grazing stock-piled native range in October and the same pasture during lush spring growth in May. In October, when cows were losing BW, they produced on average 87 g/d CH_4, whereas, on the same pasture in May, they produced approximately 252 g/d CH_4. Lassey (2007) summarized much of the CH_4 emissions data from grazing cattle determined using the SF_6 tracer technique (Johnson & Johnson, 1994).

Average CH_4 yields (30.8 - 32.7 g/kg DMI) in the present study were greater than those reported from beef and dairy cattle fed freshly cut ryegrass-based pasture (21.9-26.5 g/kg DMI; Jonker, Muetzel, Molano, & Pacheco, 2015; Jonker, Molano, Antwi, & Waghorn, 2016) and those reported for cattle grazing fresh pasture (19.1 ± 3.7 g/kg DMI; Hammond, Muetzel, Waghorn, Pinares-Patiño, Burke, & Hoskin, 2009). Results of current study in agreement with Escobar-Bahamondes et al. (2017) who used western Canadian data to model daily CH_4 emissions for different classes of cattle, lactating beef cows are estimated to produce 271 g/d and dry cows 263 g/d CH_4. However, it can be cautious to compare responses to biochar supplementation across different studies since variability can be introduced because of different feedstock sources in biochar production (Terry et al. 2019a; Winders et al. 2019).

No publication however was found regarding impact of biochar supplementation on CO_2 emissions. Chaves et al.

(2006) determined effect of pasture type on carbon dioxide production by heifers grazing alfalfa or grass pastures at three sites across western Canada. These authors (Chaves et al., 2006) measured that using the sulfur hexafluoride (SF_6) tracer technique, total carbon dioxide yield did not differ between pasture types and averaged 1.1 kg/kg DMI, respectively, which was identical with the current study.

4.4 Effect of Biochar Supplementation on Cow Ruminal SCFA, and Ammonia Nitrogen

Past research by others (Leng et al., 2012a, 2014; Saleem et al., 2018) has suggested a probable linkage between rumen microbial ecology and biochar supplementation in cattle. Therefore, in the current study, rumen fermentation parameters, including SCFA and rumen NH_3-N were measured as indicators of rumen function. As propionate is the primary glucogenic pathway and electron accepting pathway which lowers substrate availability for methanogenesis, research had attempted to develop strategies which shift rumen fermentation towards increase of propionate production (NASEM, 2016). However, a SCFA proportion shift (therefore acetate to propionate ratio) was not detected in the current study.

To our knowledge, this is the first study that has measured ruminal fermentation responses in terms of ruminal SCFA and ammonia nitrogen level of beef cows receiving a biochar supplement, therefore no comparison was available in literature. The experiment by Terry et al. (2019a) demonstrated that measures of ruminal metabolism, including total SCFA production were not affected when biochar was supplemented to Angus heifers at 0, 0.5, 1.0, and 2.0% of dietary DM. Likewise, in an in vitro rumen fermentation experiment by Pereira et al. (2014), the original biomass (corn stover vs. pine) and inclusion rate (81 vs. 186 g/kg DMI) of biochar did alter SCFA concentrations, but these were not different from the control treatment. Thus, the results of the present study agreed with these studies. The ruminal NH_3-N values in the current study were within the range (3.3 to 8.5 mg/dL) previously cited (Kang-Meznarich & Broderick, 1980) as optimum for rumen fermentation and greater than 5 mg/dL NH_3-N, which is reported to be adequate for optimal microbial protein synthesis (Satter & Slyter, 1974). The results of the current study were, also, consistent with McFarlane et al. (2017) who examined three different biomass sources of biochar, chestnut oak, yellow popular, and white pine, processed to two different particle sizes [fine (<178 μm) and coarse (>178 μm)] at 81 g/kg DMI in an orchardgrass-based diet in vitro and observed that acetate, propionate, and butyrate productions were not affected by biomass source or particle size.

4.5 Effect of Biochar Supplementation on Fecal Egg Counts and Fecal Nutrient Composition

Fecal egg counts are a common diagnostic method used to assess the presence and intensity of intestinal parasites in grazing animals (Schmidt, Hagemann, Draper, & Kammann, 2019). *Eimeria* spp. is a genus of protozoan parasites that primarily infect the intestinal tract of animals, including mammals, birds, and reptiles. These parasites belong to the phylum *Apicomplexa* and are known for causing coccidiosis, a disease that can be significant in terms of economic impact in livestock farming. Our study found evidence of biochar decreasing intestinal parasites (*Eimeria* spp.) in grazing cattle and this was also consistent with previous experiments by others (Volkmann, 1935; Van, Mui, & Ledin, 2006; Watarai & Koiwa, 2008; Paraud et al., 2011). Volkmann (1935) published his findings about efficient reduction of oocyst excretion resulting from coccidiosis and coccidial infections when charcoal was fed to domestic animals. In vitro and in vivo experiments with bovine calves showed that biochar, especially in combination with wood vinegar, was able to control parasitic protozoan *Cryptosporidium parvum* infection and to stop diarrhea of calves within one day. The number of oocysts in the feces dropped significantly after a single day of feeding biochar; after 5 days no more oocysts could be found in the feces of the calves (Watarai & Koiwa, 2008). Similar results were reported when a commercial biochar wood acetic acid product (Obionekk®, Obione, Charentay, France) was tested as a feed additive in young goats (Paraud et al., 2011). The mixture administered twice or thrice daily reduced the clinical signs of diarrhea on the first day, and the oocyst shedding in the feces decreased significantly. Over the period of the study, the mortality of the young goats was 20% in the control group and only 6.7% in the treatment group that received Obionekk® three times per day. Biochar feeding in goats may also reduce the incidence of parasites such as cestode tapeworms and coccidia oocysts like *Eimeria spp.* (Van et al., 2006).

Furthermore, biochar has been reported to be an ideal amendment for manure, soil, and compost, and therefore using cattle as a vessel to incorporate biochar into manure maybe a potential strategy to improve manure quality (Schmidt et al., 2019). The C:N ratio is the proportion of organic carbon to total nitrogen of feces or organic material. The higher C:N ratio observed was more evident in the fecal samples collected from biochar supplemented cows in the present study. For biochar treatment, total C (%) was higher in the feces which was collected at the end of the trial, relative to samples collected before the trial. Since biochar is a carbon-rich product, this response for total C was expected. The C-lattice structure of biochar has been shown to remain intact as it travels through the digestive tract (Joseph et al., 2015), thereby increasing the C content in the

excreted feces. The fertilization value of biochar-amended feces (manure) was outside the scope of this experiment and no data available in current study. However, others (Joseph et al., 2015; Schmidt et al., 2019) reported that when applied to soil the dung beetles will be able to successfully move the manure through the soil horizon to increase stable C and enhance soil fertility. Therefore, we speculated that feeding biochar to grazing cows will result in positive secondary effects on soil fertility and fertilizer efficiency, thus rending biochar a potential cost-effective, multi-beneficial supplement.

5. Conclusions

The supplementation of biochar at 2.2% (0.058% of BW) of DMI, had no effect (positive or negative) on animal performance of beef cattle grazing in a meadow bromegrass-alfalfa stand when grown in western Canada. Biochar supplementation also did not alter ruminal short-chain fatty acids or ammonia nitrogen. However, biochar supplementation reduced grazing cattle fecal oocyst counts and increased total carbon and carbon nitrogen ratio in the manure. Although the effect of biochar supplementation on enteric greenhouse gas emissions was negligible, the use of biochar as a supplementation strategy for grazing beef cattle has the potential to improve animal health and may increase soil organic matter content and thus soil fertility when manure is eventually applied to soil. More systematic multi-disciplinary research is needed to arrive at generalizable recommendations.

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Authors contributions

Dr H.A. Lardner and Ms. K. Larson were responsible for study design and revising. Dr. D. Damiran was responsible for data analyzing and drafted the manuscript. All authors revised and approved the final manuscript.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

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The Publication Ethics Committee of the Canadian Center of Science and Education.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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