

ANIMAL BEHAVIOR AND COGNITION

Use of biochar by sheep: impacts on diet selection, digestibility, and performance

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Abstract

The objective of this study was to determine the influence of biochar obtained from exothermic production of lodgepole pine (*Pinus contorta*) and quaking aspen (*Populus tremuloides*) on sheep performance and diet digestibility and on preference for a ration enriched with this carbon-based material. Twenty-four lambs were housed in individual pens and assigned to one of three treatment groups (eight animals per group), where they received: 1) a 60:40 ration of alfalfa:barley (Control), 2) an isoenergetic and isonitrogenous ration with alfalfa, barley, and 2% biochar (BC), and 3) a simultaneous offer of the Control and BC rations (Choice). Lambs were exposed to two consecutive feeding periods (Period 1: 13 d and Period 2: 21 d), representing time intervals where the evolution of intake, animal performance, and rumen parameters were assessed; *in vivo* digestibility was determined during the last 5 d of the study. Ration intake did not differ among groups of lambs ($P > 0.10$), although during some days in Period 2, intake was greater for the BC and Choice groups than for the Control group ($P < 0.05$). Lambs in Choice had a lower preference for BC than for the Control ration (Period 1: $P < 0.05$; Period 2: $P < 0.10$), although they incorporated a substantial amount of BC (39 and 40%, for Periods 1 and 2, respectively) into their diets. No differences in body weight gains (ADG) or gain-to-feed ratios were found among groups of animals ($P > 0.10$), although dry matter digestibility and digestible dry matter intake was greater for lambs in the BC group than for lambs in Control group ($P < 0.05$). The ruminal concentration of the volatile acid acetate in Period 2 was greater for BC than for Choice ($P < 0.05$). During the same period, the concentration of valerate and ruminal pH values were greater in BC than in Control ($P < 0.05$). Thus, the addition of biochar to grain-based diets enhanced diet digestibility and influenced some ruminal parameters in lambs. Nevertheless, these positive effects were not reflected in significant improvements on ADG or feed conversion efficiencies. Lambs offered choices between Control and BC rations formed a diet with concentrations of biochar of ~1.2%, suggesting that these animals would tolerate such levels without reductions in ration palatability.

Key words: diet selection, digestibility, feed efficiency, feed intake, lamb

Introduction

Biochars are pyrolysis-based products intended for use as a soil amendment to increase soil fertility, with positive effects on field water holding capacity, pH, cation exchange capacity,

nutrient availability, and fertilizer use efficiency (Atkinson *et al.*, 2010; Manyà, 2012; Knox, 2018). The ability of biochars to bind to different chemicals (Thies and Rilling, 2012) and to adsorb microorganisms (Leng, 2014) and toxins (Toth and Dou, 2016) make these substances appealing for their use in animal

Abbreviations

ADF	acid detergent fiber
ADFD	ADF digestibility
ADL	acid detergent lignin
ADG	average daily gain
BUN	blood urea nitrogen
BW	body weight
CP	crude protein
DE	digestible energy
DM	dry matter
DMI	dry matter intake
DDMI	digestible dry matter intake
DMD	dry matter digestibility
FO	fecal DM output
NDF	neutral detergent fiber
NDFD	neutral detergent fiber digestibility
LSMeans	least squares means
VFA	volatile fatty acids

feeding systems to enhance animal performance and reduce environmental impact. For instance, some studies have shown that the addition of biochar to ruminant diets (~1% of the diet) lead to reductions in methane emissions in vitro (Hansen et al., 2012; Leng et al., 2012a; Saleem et al., 2018) and in vivo (Leng et al., 2012b, Winders et al., 2019), which attenuates environmental impacts and increases the efficiency of nutrient use by ruminants (Silivong and Preston, 2015). The mechanism underlying this process has been attributed to the electrical conductivity and electron-buffering capacity of carbonaceous materials such as biochars (Yu et al., 2015), which enhance the redox reactions that take place in the rumen, increasing the energy conversion efficiency in livestock (Leng et al., 2013). Interactions of biochar surfaces with rumen microorganisms that enhance the efficiency of energy production and utilization have also been discussed (Leng et al., 2012a,b). In addition, since most biochars are alkaline in nature, they may buffer rumen pH, resulting in improved livestock weight gains under high-energy-containing diets (Kammann et al., 2017).

Despite all the aforementioned benefits, much of the scientific information available on biochars and livestock feeding comes from materials produced in Eurasia and Oceania or from in vitro studies. Information about in vivo utilization of biochars produced from the combustion of resources in North America (e.g., trees such as lodgepole pine, aspen, or olive) and fed to sheep are limited, particularly when feeding high concentrate diets (Winders et al., 2019). In addition, it is unknown whether livestock can self-select biochar given the potential benefits of this substance on ruminant nutrition and productivity. If ruminants learn to ingest biochar, then they will select the product based on their specific and dynamic needs, which may enhance their nutrition and welfare. Thus, the objective of this study was to determine the impact of biochar obtained from the exothermic production of lodgepole pine (*Pinus contorta*) and quaking aspen (*Populus tremuloides*) on sheep performance and diet digestibility and on preference for a ration enriched with this carbon-based material.

Materials and Methods

Animals and management

The study was conducted at the Green Canyon Ecology Center, Utah State University, located in Logan, UT (41°44'76"N;

–111°50'3.80"W) between 28 May and 24 July 2018, according to experimental procedures approved by the Utah State University Institutional Animal Care and Use Committee (approval no. 10032). Throughout the study, lambs had free access to culinary water and trace mineral salt blocks.

Twenty-four 3-mo-old Finn-Columbia-Polypay-Suffolk crossbred lambs of both sexes with an average initial body weight (BW) of 37.9 ± 0.8 kg (mean ± SEM) were individually penned outdoors under a protective roof in individual adjacent pens with dirt floors, measuring 2.4 × 3.6 m. All animals were orally dosed against gastrointestinal parasites (Albendazole [Spectrum Pharmaceuticals, Cambridge, MA] at 7.5 mg/kg BW) and vaccinated with *Clostridium perfringens* type C and D toxoid (Zoetis Animal Health, Fairfield, NJ). Lambs were fed ad libitum amounts of alfalfa pellets and 300 g of rolled barley (as-fed basis)/animal/day as their basal diet for 14 d as an adaptation period to their pens and feeding conditions.

Biochar production

Biomass from whole trees, including limbs and needles, of lodgepole pine (*Pi. contorta*) and quaking aspen (*Po. tremuloides*) were pyrolyzed by commercial biochar producers Biochar Solutions Inc. and Confluence Energy (Kremmling, CO) through their proprietary two-stage process. In the first stage of the process, the material was carbonized in an oxygen limited environment at temperatures between 500 and 700 °C for less than 1 min. In the second stage, the material was held in a hot gas environment with no oxygen for up to 14 min at temperatures between 300 and 550 °C, and then cooled to ambient temperature. The preparation process results in two size fractions of biochar of different particle size: 1) approximately 80% of material is 1.5 cm long by 1 cm wide by 0.5 cm thick, and about 20% of the material is a fine dust fraction in the order of 10 to 100 s of microns. Given this wide distribution in size, the material used for the study was biochar that passed through a 1-mm screen.

It is noted that biochar is not an approved feed ingredient in the United States, although chemical analyses support its safety for use as a feed ingredient. The biochar was characterized by Control Labs, Watsonville, CA, using the International Biochar Initiative Laboratory tests for certification program. Methods 3050B/6020 (US EPA, 1996, 1998a) were used to measure concentration of arsenic, cobalt, molybdenum, sodium, and selenium in the biochar, which were all nondetectable with detection minimums of 0.46, 0.46, 0.46, 459.1, and 0.92 mg/kg, respectively. Method 7471 (US EPA, 1998b) was used to measure concentration of mercury, which was also nondetectable with a detection minimum of 0.001 mg/kg. Cadmium and lead concentration were detected at 0.2 and 2.0 mg/kg (detection minimum 0.18; methods 3050B/6020; US EPA, 1996, 1998a). The biochar had 92.6% dry matter (DM), and a composition of 82.7% carbon, 0.6% total nitrogen, and 99.3% organic matter on a DM basis (ASTM D1762-84), with a pH value of 8.36.

Treatments and experimental design

Lambs were randomly assigned to three treatment groups (N = 8 lambs per group) and offered ad libitum amounts of the following rations: 1) Control, a basal diet of alfalfa hay and barley (60:40; Table 1); 2) Biochar (BC), a isocaloric and isonitrogenous basal diet containing alfalfa, barley and 2% biochar (Table 1), and 3) Choice (CH), lambs had a simultaneous offer of ad libitum amounts of the Control and BC rations. The two rations used in the study were designed to minimally affect crude protein (CP)

and digestible energy (DE) concentration (Table 1). Rations were ground to a particle size of 1 to 3 mm to reduce sorting and they were prepared by mixing the ingredients into batches that were fed to animals during periods of 7 to 10 d, when new batches were made.

Lambs were randomly distributed across groups and pens, considering the variation of gender (female and wethers) and weight, resulting in a uniform distribution of animals within each treatment group. Lambs in the Control, BC, and CH groups had initial BW of 37.9 ± 1.5 ; 38.0 ± 1.4 ; and 37.8 ± 1.4 kg, respectively.

Exposure to the rations involved a period of familiarization, and then two periods of measurements (Periods 1 and 2). Intake was measured on a daily basis during Periods 1 and 2, whereas rumen fluid and blood samples were collected at the end of each of these periods. Samples of refusals and feces were collected during the last 5 d of Period 2 for fecal output and digestibility estimates (see below).

Familiarization period

All lambs were exposed to a familiarization period, where the Control and BC groups received their respective diets in ad libitum amounts from 0700 to 1600 for eight consecutive days (12 June to 19 June 2018). The amounts offered were adjusted every morning, so that animals had at least 20% of refusal in their feeders on the ensuing day at 0700. The Choice group was offered the BC diet on even-numbered days and the Control diet on odd-numbered days. Refusals were collected at 1600 and weighed, and no other feed was offered until the following day.

Periods of measurements

The three groups received their respective diets in ad libitum amounts from 0700 to 1600, as described for the familiarization period. Rations were offered in two separate wooden containers for the Control and BC groups. For the CH group, rations were also presented in two separate containers, but each container had a different ration (Control and BC) and the placement of the feed in each container (left vs. right) was randomized across days. The amounts offered for each ration were adjusted

every morning, so that animals had at least 20% of refusal in their feeders on the ensuing day at 0700 to ensure that lambs consistently selected a ration rather than an eating position.

To assess the evolution of intake, animal performance, and rumen parameters across time, there were two periods of exposure to the diets. Period 1 occurred from 20 June to 2 July 2018 (13 d), and Period 2 occurred from 3 July to 23 July (21 d).

Measurements

Intake

Daily ration intake was measured by the difference between the amounts of ration offered and refused. Dry matter intake (DMI) was expressed as g DM consumed/kg BW. For the Choice group, ration preference by lamb was estimated as the daily proportion of the DMI, calculated for each ration (Control and BC), relative to the total amount of DMI.

Animal growth and feed efficiency

Lambs were weighed, with a previous liquid and solid fast period of 12 h. Average daily weigh gains (ADG) were estimated as the weight gained during a period divided by the number of days that elapsed during that period. The animals were weighed the day before the familiarization period had started (11 June 2018) and at the end of Periods 1 (3 July 2019) and 2 (24 July 2019). Intake and gain data were used to estimate feed conversion efficiency as gain-to-feed ratio (kg of BW gain:kg of DM intake) for Periods 1 and 2 of the study.

Nutritional analyses

Samples (50 g) of each of the rations were taken on a daily basis and then composited by period. During the last 5 d of the study (Period 2; 19 July to 23 July), representative (20% of the amount retrieved) daily samples of refusals were collected, as well as daily fecal grab samples (20 to 30 g DM) from each lamb in the study, retrieved between 0800 and 0900. These samples were used to determine fecal DM output and in vivo digestibility during that period (see below).

Samples of feed, orts, and feces from each lamb were placed in plastic seal top bags, labeled and immediately stored in a freezer at -20 °C. Samples were subsequently freeze dried (Free Zone 18 Liters, Labconco Corporation, Kansas City, MO) at -60 °C until two consecutive weights did not differ in a 24-h period, and subsequently ground to pass the 1-mm screen of a Wiley mill (model 4; Thomas Scientific Swedesboro, NJ). Feed, orts, and fecal samples were then composited by lamb over the 5-d sampling period.

All composited samples were analyzed for DM, CP, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) contents. DM was determined by drying the samples at 105 °C for 3 h in a forced-air drying oven (Shreve et al., 2006). CP was calculated by analyzing the N concentration of the samples using a Leco FP-528 nitrogen combustion analyzer (AOAC, 2000; method 990.03) and applying the 6.25 conversion factor (Mossé, 1990). NDF contents were measured according to Van Soest et al. (1991; Procedure A), and ADF contents were measured according to AOAC (1990; Method 973.18). ADL was determined following the procedures described by Robertson and Van Soest (1981). Composited samples for rations across periods were also analyzed for NDF digestibility at 30 h (NDFD 30h) using the Daisy^{II} system (Damiran et al., 2008). The DE of the rations was calculated according to NRC (1985).

Fecal DM output and in vivo digestibility

Fecal DM output (FO) during Period 2 was determined using the concentration of an internal marker, ADL, in the ration

Table 1. Nutritional characteristics (% of DM) of the rations used in the study

	Control	BC ¹
	g/kg, as-fed basis	
Ingredient ²		
Alfalfa	60	54.5
Barley	40	43.5
Biochar	—	2
Composition of the diets	DM basis	
CP, %	15.6 ± 0.2 ³	14.8 ± 0.4
ADF, %	27.7 ± 1.3	26.3 ± 0.8
NDF, %	34.8 ± 0.7	33.4 ± 0.4
ADL, %	6.4 ± 0.3	6.7 ± 0.3
NDFD (30 h), ⁴ %	13.5 ± 0.7	12.7 ± 0.5
NDFD (30 h)_NDF, ⁵ %	38.9 ± 1.9	37.9 ± 1.2
DE, ⁶ Mcal/kg	3.05	3.05

¹BC, diet containing biochar.

²All ingredients were ground to a particle size of 2 to 4 mm.

³SEM composited across two periods (P1 and P2).

⁴In vitro NDF digestibility at 30 h.

⁵NDFD as a percentage of NDF.

⁶Calculated based on values obtained from NRC (1985).

consumed and in feces (Van Soest, 1994). Fecal output was then determined using the following formula: FO (g/d) = [DMI (g/d) × ADL in feed (g/g)]/ADL in feces (g/g) (Cochran and Galyean, 1994). Once fecal output was determined, dry matter digestibility (DMD) was calculated for each lamb as follows: DMD (%) = [(DMI (g/d) – FO (g/d))/DMI (g/d)] × 100 (Cochran and Galyean, 1994).

Daily intakes and fecal outputs of NDF, ADF, and N during Period 2 were used to calculate their apparent total tract digestibility (NDFD, ADFD, and ND, respectively). Digestible DMI (DDMI) was calculated as the product of DMI (g/d) and DMD.

The nitrogen excreted through the feces (g/lamb) was calculated by multiplying the fecal output by the nitrogen concentration in feces.

Rumen and blood analyses

During the last day of each sampling period, rumen fluid was collected with a stomach tube and a vacuum pump at 3 h after food distribution in the morning. The first 30 to 40 mL of rumen fluid collected from each lamb was discarded to avoid salivary contamination. Ruminal fluid was strained through four layers of cheesecloth, and its pH was immediately measured (pH meter no. 44, Beckman Instruments, Palo Alto, CA). Samples of 18 mL were added to vials containing 2 mL of 6 M HCl and stored at –20 °C before analyses for volatile fatty acids (VFA). Before analyses, samples were centrifuged at 20,000 × g for 20 min. Concentrations of VFA were determined using a gas chromatograph (Perkin ElmerAutoSystem gas chromatograph, Bridgeport Avenue, Shelton, CT) on a Restek column packed with Stabilwax-DA. Nitrogen was used as a carrier gas at 150 kPa. The oven temperature was 125 °C, and the detector, injector, and column temperature were 100 °C.

Blood samples (without EDTA added; Becton Dickinson Vacutainer System; Becton Dickinson and Company, Franklin Lakes, NJ; 10-mL serum vacutainer tubes) were collected via jugular venous puncture during the last day of each sampling period 3 h after food distribution in the morning. Samples were allowed to clot for 45 min before being centrifuged (1,500 rpm for 15 min). The serum was extracted, placed in 1.5-mL microcentrifuge tubes, and immediately submitted to the Utah Veterinary Diagnostic Laboratory (Logan, UT) for blood urea nitrogen (BUN) analyses. The assay was performed with a Siemens Dimension Xpand Plus analyzer (Siemens Healthcare Diagnostics, Newar, DE) using Siemens urea nitrogen flex reagent, in an enzymatic method, which uses urease enzyme in a bichromatic rate technique.

Statistical analyses

Ingestive, performance, and physiological response variables were analyzed as a split-plot in time design with lambs (random factor) as the whole plot nested within Group (Control, BC, and Choice) as the whole-plot factor. Day (response variables: intake, digestibility, fecal output) or Period (response variables: ADG, efficiency, rumen, and blood parameters) were the split-plot factors (repeated measures). The mixed model formulation was as follows: $Y_{ijk} = \mu + \alpha_i + \eta k_{(i)} + \beta_j + (\alpha\beta)_{ij} + \varepsilon k_{(ij)}$, where Y_{ijk} = response variable, μ = mean, α_i = fixed effect of Group, β_j = fixed effect of time, $(\alpha\beta)_{ij}$ = interaction between Group and time, $\eta k_{(i)}$ = whole-plot error, and $\varepsilon k_{(ij)}$ = split-plot error (Littell et al., 1998; Wang and Goonewardene, 2004).

Separate analyses were conducted for the CH group to estimate lamb intake, where animal was the whole plot, ration (Control or BC) was the whole-plot factor, and day was the split-plot factor (repeated measure). Percentage of preference for each ration [(intake of a ration/total ration intake) × 100] was

analyzed with day and period and their interaction as fixed factors and lamb and period × lamb as the random factors. Radial smoothing of the repeated measures on each lamb at each period was applied. The 95% CI of the estimated percentage was used to determine the range in which the true average proportion selected could vary. A preference was considered when the CI for the ration was greater than a theoretical mean of 50% (i.e., equal or indifferent preference), and when it did not include 50%.

All analyses were computed using the Mixed procedure of SAS (SAS Inst., Inc. Cary, NC; Version 9.4 for Windows). Least squares means (LSMeans) were compared pairwise using the Least Significant Difference test when F-ratios were significant ($P < 0.05$) and reported along with their standard errors (SEM). A tendency was considered when $0.10 > P > 0.05$.

To adjust the temporal autocorrelation observed in data measured on time, covariance structures were tested to fit the models, based on Akaike's information criterion, where the smallest value represents the best model adjusted.

The normal distribution of data was tested by Kolmogorov–Smirnov test ($P > 0.05$). Data were transformed when necessary (preference, feed efficiency) by the logarithmic function to meet homogeneity of variance assumptions, and back transformed to report LSMMeans.

Results

Diet quality

The average composition of the rations used in the study is presented in Table 1. Rations (Control and BC) had similar concentrations of CP, fiber, and lignin, and were isocaloric.

Familiarization period

During the 8-d familiarization period, there were no differences in ration intake among groups of lambs (33.7 [Control], 34.5 [BC], and 30.2 g/kg BW [Choice]; SEM = 2.7 g/kg BW; $P = 0.465$; 1.3 [Control], 1.3 [BC], and 1.2 kg/d [Choice]; SEM = 0.1 kg/d; $P = 0.342$), and no Group × Day interaction ($P > 0.10$) was detected, although ration intake increased across days for all groups (Day effect; $P < 0.0001$).

Ration intake, animal growth, and feed efficiency

There were no differences in average ration intake for the three groups of lambs tested, either during Period 1 (Group effect; $P = 0.545$; Day effect; $P = 0.014$; Group × Day; $P = 0.944$; Figure 1A) or during Period 2 (Group effect; $P = 0.186$; Day effect; $P = 0.001$; Figure 1A). A Group × Day interaction ($P = 0.045$) was detected for Period 2 given that during some days, the Control group ate less than the BC and/or the Choice groups ($P < 0.05$; Figure 1B).

Lambs in the choice group ate on average more Control than BC ration during both periods of the study (Ration effect; $P = 0.01$ and $P = 0.04$; Ration × Day; $P = 0.729$ and $P = 0.106$, for Periods 1 and 2, respectively; Figure 1A).

Preference for BC was on average $39 \pm 4\%$ (with 95% CI of [29, 50%]) and $40 \pm 4\%$ (with 95% CI of [30, 51%]) for Periods 1 and 2, respectively. The estimated difference from 50% was $-10 \pm 4\%$ with 95% CI of (–19, –1%) for Period 1, indicating significantly lower preference than 50% ($P = 0.038$). The estimated difference was $-9 \pm 4\%$ with 95% CI (–18, 0%) with a trend for a lower preference than 50% ($P = 0.051$).

No differences were detected in ADG among groups of lambs for any of the periods assayed or for the two combined periods (Group effect; $P = 0.839$; Period effect; $P = 0.392$; Group × Period;

$P = 0.576$; Figure 2A). Likewise, gain-to-feed ratios did not differ among groups of lambs (Group effect; $P = 0.502$; Group \times Period; $P = 0.334$; Figure 2B). Nevertheless, ADG and feed conversion efficiencies during Period 1 were 23 and 28% greater, respectively, for the BC than for the Control group (Figures 2A and 2B).

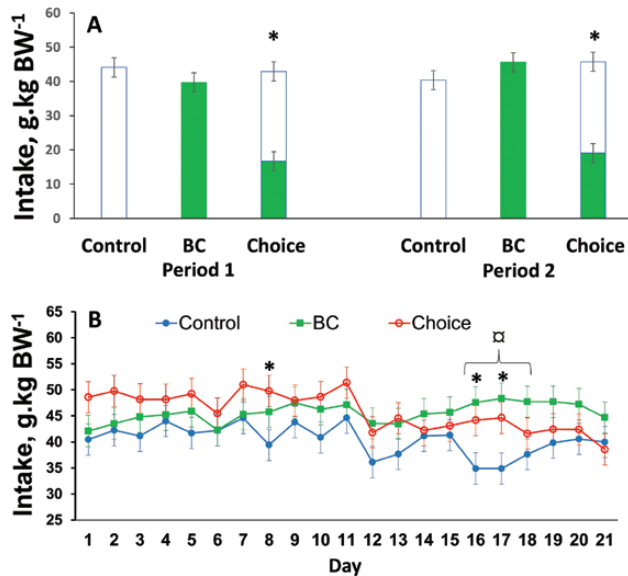


Figure 1. (A) Average intake by three groups of lambs offered three different rations: 1) Control: alfalfa:barley (60:40), 2) BC: control containing 2% of biochar, and 3) Choice: lambs had a choice between Control and BC. Lambs were fed the rations during two consecutive periods (Periods 1 and 2). *Lambs in Choice ate more Control than BC ration ($P < 0.05$). (B) Daily intake by the three groups of lambs during Period 2, where a Group \times Day interaction was detected ($P = 0.045$). *Choice had greater ration intake than Control ($P < 0.05$). *BC had greater ration intake than Control ($P < 0.05$). Values are means for eight animals per group. Vertical bars represent \pm SEM.

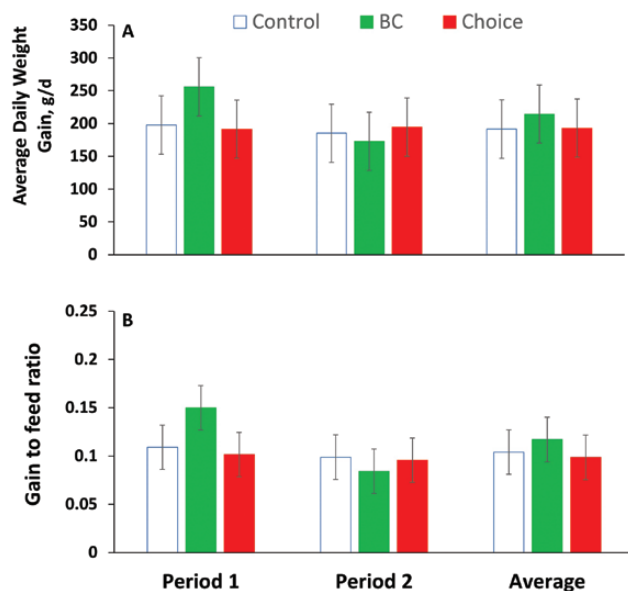


Figure 2. (A) Average daily weight gains and (B) gain-to-feed ratios by three groups of lambs offered three different rations: 1) Control: alfalfa:barley (60:40), 2) BC: Control containing 2% of biochar, and 3) Choice: Lambs had a choice between Control and BC. Values are means for eight animals per group. Vertical bars represent \pm SEM.

Fecal DM output and in vivo digestibility

No differences in fecal output ($P = 0.359$), excreted N ($P = 0.643$), N ($P = 0.278$), or NDF ($P = 0.275$) digestibilities were detected among groups of lambs (Table 2). However, lambs in the BC group had greater levels of DMD than the Control group ($P = 0.013$), and DMD in BC tended ($P = 0.07$) to be greater than in the Choice group (Table 2). Likewise, the BC group had greater DDMI than the Control group ($P = 0.003$; Table 2). The greatest values of ADFD were observed for lambs consuming the Control diet ($P = 0.009$; Table 2). No Day effect or Group \times Day interactions ($P > 0.10$) were detected for fecal output or any of the digestibility estimates.

Blood and rumen determinations

During Period 2, an increment in the ruminal concentrations of the VFAs acetate (Group \times Period; $P = 0.014$) and propionate (Group \times Period; $P = 0.031$) was observed for the group BC relative to Period 1 (Table 3). In contrast, the group Choice revealed a tendency for the opposite pattern (acetate: $P = 0.102$; propionate: $P = 0.061$), and no differences between periods were observed in the Control group for these variables ($P > 0.10$; Table 3). The concentration of acetate in Period 2 was greater for BC than for Choice ($P = 0.040$) and it was similar between BC and Control groups ($P = 0.132$) during the same period. A decline in the concentration of valerate was observed for the Control group (Group \times Period; $P = 0.035$) and in ruminal pH values (Group \times Period; $P = 0.046$) in Period 2 relative to Period 1 (Table 3). During Period 2, the concentration of valerate ($P = 0.039$) and ruminal pH values ($P = 0.049$) were greater in BC than in Control (Table 3).

Discussion

Intake and preference

The present study reveals that the addition of biochar (2%) to an alfalfa:barley ration (BC), or the simultaneous offer of rations with or without biochar (Choice) did not modify feed intake relative to the offer of a ration without biochar (Control). Previous studies using lesser or greater concentrations of biochar than those offered to the group BC yielded similar results. For instance, DMI was not affected when biochar was present in the diet of growing cattle (0.8% to 3%; Winders et al., 2019; 0.6% Leng et al., 2012b) or goat diets (1%, Silivong and Preston, 2015), although biochar added at a rate of 0.8% stimulated intake in a finishing cattle diet (Winders et al., 2019). Likewise, intake by dairy cows was enhanced with the addition of 20 or 40 g/d of activated carbon to poor-quality corn silage diets containing mycotoxins, as activated carbon bind to these toxins and attenuate their negative postingestive effects (Erickson et al., 2011). Beyond the potential actions as a toxin binder, the mechanism by which biochar may enhance feed intake in livestock is not completely understood but it may involve providing appropriate microenvironments for an improved microbial habitat, in particular by a substrate with a large surface area to weight ratio (Leng et al., 2012a, b). Increased microbial activity may entail an enhanced rate of passage through the gastrointestinal tract and thus greater levels of feed intake. Such effect may be more evident when diets are of lower nutritional quality than those offered in the present study (alfalfa, barley), where rates of passage are already high without the addition of biochar. Nevertheless, during some days in Period 2 of the study, it was evident that intake by the BC and Choice groups was greater than intake by the Control group.

Lambs offered a choice between the Control and BC rations preferred the feed without biochar (~60%). These results are

Table 2. Dry matter, NDF, ADF, and N digestibilities, intake and fecal output by three groups of lambs (N = 8) fed three different rations: (1) Control: alfalfa:barley (60:40), (2) BC: Control containing 2% of biochar, and (3) Choice: Lambs had a choice between Control and BC

Item	Control	BC	Choice	SEM ¹	P-value ²
DMD, %	57.0 ^b	60.7 ^a	58.5 ^{ab}	0.80	0.013
NDFD, %	26.8	29.3	26.5	1.30	0.275
ADFD, %	26.6 ^a	21.2 ^b	19.6 ^b	1.50	0.009
ND, ³ %	53.0	58.4	53.4	2.58	0.278
DMI, ⁴ g/kg BW ⁻¹ ·d ⁻¹	41.9	43.3	44.5	2.19	0.692
DMI, ⁵ kg/d	1.87	1.93	1.85	0.19	0.960
DDMI, g/kg BW ⁻¹ ·d ⁻¹	19.5 ^b	26.4 ^a	22.8 ^{ab}	1.24	0.003
Fecal output, g/kg BW ⁻¹ ·d ⁻¹	15.0	17.1	16.2	1.03	0.359
Excreted N, g kg BW ⁻¹ ·d ⁻¹	0.36	0.39	0.40	0.03	0.643

¹Standard error of the group mean.

²Fixed effect of group.

³ND, nitrogen digestibility.

⁴Average intake over two consecutive periods.

⁵Average intake over two consecutive periods.

^{a,b}Means in a row with different letters differ (P < 0.05).

consistent with previous research showing that dairy cows reduce their preference for rations that contain activated carbon (10 to 80 g/d; Erickson et al., 2011). Despite lower preference, lambs incorporated a significant proportion of BC (~40%) into their diets, representing 1.2% of biochar in the mixed ration ingested, a concentration closer to what has been typically used (i.e., 0.6% to 1%) in in vitro and in vivo studies. Thus, even when the biochar-containing ration was less preferred than the Control ration, lambs incorporated significant amounts of biochar into their diet, which were likely beneficial at those rates. Collectively, animals in the Choice group did not prefer the BC ration but their selection diluted the concentration of biochar in their diet, a behavior that led to some improvements in diet digestibility and that modified some ruminal parameters. Foraging preferences in ruminants are triggered by learning mechanisms that integrate the feeds' oro-sensorial characteristics with their postingestive consequences (Provenza, 1995, 1996), such that those feeds that promote a homeostatic utility to the animal are ingested and those that reduce fitness are avoided. Nevertheless, no clear improvements in diet digestibility or performance were observed for lambs of the Choice group relative to lambs in the Control group (see below). However, potential aversive and stressful states triggered by ingesting the same ration and flavors too frequently or in excess might have been less prevalent in the Choice group, given that sheep are generalist herbivores that evolved ingesting diverse diets composed of different flavors and chemicals (Provenza, 1996). Sheep consuming a diversity of feeds had lower levels of stress than sheep exposed to monotonous rations (Catanesi et al., 2013). Similarly, sheep in the Choice group experienced a greater diversity of flavors in their diet, and thus a greater degree of sensorial benefits, provided by the addition of charcoal to one of the rations on offer.

Animal weights, feed efficiency, and digestibility

Previous research has shown that biochar enhances BW gains and feed efficiency in cattle (Leng et al., 2012b) and goats (Silivong and Preston, 2015). Nevertheless, the present study

Table 3. Rumens VFA, BUN, and rumen fluid pH after 13 (Period 1) and 21 days (Period 2) of feeding three groups of lambs (N = 8) with three different rations: (1) Control: alfalfa:barley (60:40), (2) BC: Control containing 2% of biochar, and (3) Choice: Lambs had a choice between Control and BC

Item	Control						Choice						SEM ¹	P-value ²	P-value ³	P-value ⁴		
	Period 1		Period 2		Period 1		Period 2		Period 1		Period 2							
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2						
VFA (mmol/L)																		
Acetate	76.2 ^a	113.8 ^{bc}	107.2 ^b	83.9 ^{ab}	96.5 ^{ab}	92.4 ^{ab}	31.5 ^a	31.5 ^{ab}	42.2 ^{ab}	33.1 ^{ab}	35.0 ^{ab}	2.70	2.70	2.86	2.63	2.63	0.667	0.014
Propionate	31.5 ^a	42.6 ^b	42.2 ^{ab}	31.5 ^{ab}	33.1 ^{ab}	35.0 ^{ab}	2.38	2.70	2.50	2.86	2.63	18.7	17.2	23.2	20.4	20.4	0.830	0.031
Acetate:propionate	16.2	18.7	22.6	17.2	23.2	20.4	0.5	0.6	0.7	0.7	0.5	0.4	0.4	0.3	0.4	0.4	0.758	0.374
Butyrate	0.5	0.7	0.7	0.6	0.7	0.5	0.1	0.4	0.3	0.3	0.4	2.4 ^{ab}	2.4 ^{ab}	3.2 ^a	2.0 ^b	2.0 ^b	0.168	0.153
Isobutyrate	0.1	0.4	0.3	0.4	0.3	0.4	179.1	135.7	176.0	157.0	150.5	3.1 ^a	3.1 ^a	3.2 ^a	2.0 ^b	2.0 ^b	0.984	0.115
Isovalerate	2.4 ^{ab}	3.1 ^a	3.2 ^a	2.4 ^{ab}	3.2 ^a	2.0 ^b	19.1	20.3	20.8	21.1	17.8	127.0	135.7	157.0	150.5	150.5	0.517	0.397
Valerate	127.0	179.1	176.0	135.7	157.0	150.5	21.1	20.3	20.8	21.1	17.8	19.1	20.3	21.1	17.8	17.8	0.815	0.035
Total	21.1	19.1	20.8	20.3	21.1	17.8	5.8 ^{ab}	6.0 ^a	5.9 ^{ab}	6.0 ^a	5.7 ^b	14.5	14.5	14.5	14.5	14.5	0.980	0.020
BUN (mg/dL)	5.8 ^{ab}	6.0 ^a	5.9 ^{ab}	5.9 ^{ab}	6.0 ^a	5.7 ^b	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.783	0.145
pH	6.0 ^a	6.0 ^a	6.0 ^a	6.0 ^a	6.0 ^a	6.0 ^a	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.869	0.046

¹Standard error of the group mean.

²Fixed effect of group.

³Fixed effect of period.

⁴Group × period effect.

^{a,b,c}Means within a row with different superscripts differ (P < 0.05).

did not find significant differences among groups of lambs for these parameters, an outcome that was probably influenced by a high variability among individuals, particularly during Period 1 of the study. During this period, ADG and feed conversion efficiencies were 23 and 28% greater, respectively, for the group that ingested the biochar-containing ration (BC) than for the group that received the ration without biochar (Control), although differences were non-significant. It is also possible that the type of biochar used in the current study presented different characteristics than those used in previous research where positive responses on efficiency and body weight gains were found (Leng et al., 2012b; Silivong and Preston, 2015). For instance, different types of biochar of known and unknown sources significantly varied in their efficacy at reducing methane production during *in vitro* studies (Leng et al., 2013). The concentration of biochar in the ration might have also been a factor that constrained differences in ADG and efficiencies among groups. Silage mixtures containing 8.8% and 16.6% of biochar led to increments in total VFA production (Calvelo Pereira et al., 2014), which suggests increased microbial fermentation (Guan et al., 2008) at those higher concentrations of biochar. Finally, the length of exposure to the rations might have not been long enough to reveal performance differences among treatments. Nevertheless, growing sheep in other studies (e.g., Poli et al., 2018) change their performance responses to dietary treatments within the time frame of the combined periods assayed in this study (i.e., 34 d).

Findings on digestibility in the present study support the notion that biochar had a positive influence on lamb nutrition, as the group that had biochar in the ration (BC) had greater values of DMD and DDMI than the group not fed biochar (Control). Nevertheless, the greatest values of ADFD were observed for the Control diet, an outcome that could be attributed to lower fermentation rates of the ADF fraction present in biochar, which was produced from woody species that typically present lower levels of ADF digestibility.

The majority of studies exploring the effects of biochar on ruminant diets involve *in vitro* testing, which do not accurately replicate what happens inside the animal, as they do not include ingestive and other physiological processes occurring *in vivo* (Villalba and Provenza, 2010). Our study and others (e.g., Silivong and Preston, 2015; Winders et al., 2019) show that addition of biochars to rations can actually enhance digestibility in ruminant animals. Biochars may enhance growth of certain rumen microbial communities by providing solid surface areas where microorganisms could efficiently transfer substrate and improve the efficiency of ATP production, increasing feed digestibility and digestion efficiencies (Leng, 2013). In addition, biochar surfaces contribute to enhance populations of methanotrophic relative to methanogenic microbes (Feng et al., 2012), reducing the production of methane, which leads to improvements in feed efficiency (Leng et al., 2012a,b, 2013). Propionate to acetate ratios also increase with such improvements in efficiency (Shabat et al., 2016), but no significant differences among groups were detected for this parameter in the present study, although those ratios were ~13 to 17% greater for the Choice and BC groups than for the Control group during Period 1.

The concentration of acetate in Period 2 was greater for BC group than for Choice group, and it tended to be greater for BC group than for the Control group during the same feeding period, suggesting not only greater active bacterial fermentation and host rumen epithelial absorption for the BC group (Guan

et al., 2008), but also greater production of methanogenic substrates (Danielsson et al., 2017). Concentrations of acetate and propionate were more variable across periods for groups that received biochar for BC and Choice groups than for the Control group.

During Period 2, the concentration of valerate was greater in BC than in Control (Table 3), and significantly greater concentrations of rumen butyrate and valerate have been found to be related to greater feed conversion efficiencies in steers (Guan et al., 2008). During the same period, ruminal pH values were greater in BC than in Control, suggesting that biochar may have prevented the decrease in pH due to several days of feeding with high concentration of grain, which was made evident in Period 2. This pattern could be attributed to the fact that most biochars are alkaline (Kammann et al., 2017), a benefit particularly relevant when diets contain high concentration of grain, such as those offered in the present study. Biochar alkalinity is defined as the capacity of biochar to accept protons without significantly altering its chemical structure (Fidel, 2012). Biochar alkalinities vary widely among biochars, mostly given by (1) organic functional groups (0.03 to 0.92 mEq protons accepted/g), (2) carbonates (0.02 to 1.5 mEq protons accepted/g), and (3) other inorganic groups (0.00 to 0.26 mEq protons accepted/g), and with pKa values > 5 (Fidel et al., 2017).

Finally, and in contrast to other studies (Silivong and Preston, 2015) where a positive effect of biochar was found on N retention, no effect was observed of biochar on nitrogen digestibility or BUN values. Previous *in vitro* studies also report no effects of different sources of biochar on ammonia production (Calvelo Pereira et al., 2014).

In conclusion, the addition of biochar to alfalfa:barley diets enhanced diet digestibility and influenced some ruminal parameters in sheep, some indicative of positive effects on lamb nutrition, although increments in the VFA acetate for the BC treatment may suggest increments in methane production and reductions in microbial efficiency (Johnson and Johnson, 1995). Biochar addition to the ration or self-selection of the biochar-containing ration did not improve ADG or feed conversion efficiencies, although these parameters were enhanced over 20% (Period 1) in animals fed the biochar-containing ration. Longer periods of exposure to biochar than those provided in this study may be needed for observing significant differences in performance responses.

Lambs offered a choice between rations with or without biochar preferred the later, although they incorporated substantial amounts of biochar-containing rations into their diets (40%), suggesting that these animals would tolerate biochar at 1.2% without reductions in ration palatability.

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Conflict of interest statement

None declared.

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