Small Ruminant Research 183 (2020) 106042

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Effects of biochar produced from different biomass sources on digestibility, ruminal fermentation, microbial protein synthesis and growth performance of male lambs

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ARTICLE INFO

Keywords:
Biochar
Fattening
Microbial protein
Rumen fermentation
Sheep

ABSTRACT

Twenty four Kermanian ram lambs (21.90 ± 2.24 kg of body weight) were used in a completely randomized design to determine the effects of the addition of walnut shell biochar, pistachio by-product biochar, and chicken manure biochar on feed intake, nutrient digestibility, ruminal fermentation parameters, microbial nitrogen supply (MNS), and growth performance in a 90-day experimental period. Lambs were randomly allocated to one of four experimental diets containing 0 (no added biochar; control), 1 % walnut shell biochar (WSB), 1 % pistachio by-product biochar (PBB), and 1.5 % chicken manure biochar (CMB). Addition of WSB, PBB, and CMB to the diet had no effect on dry matter intake (DMI), but improved ($P < 0.01$) average daily gain (ADG) and feed conversion ratio (FCR) compared to control diet. Apparent total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) were increased ($P < 0.01$) with addition of WSB as compared to control. Ruminal ammonia-N (NH_3-N) was higher in lambs fed either WSB or PBB compared to control ($P < 0.05$). The pH values, number of rumen protozoa, total volatile fatty acid (VFA), and molar proportion of individual VFA were not affected by treatments. Concentration of allantoin, xanthine plus hypoxanthine, and total purine derivatives (TPD) in the urine and estimated microbial nitrogen supply (MNS) were increased with addition of biochar from all sources ($P < 0.05$). These results indicate that biochar can be incorporated in fattening lamb diets as a low cost feed additive in order to manipulate ruminal fermentation and improve feed efficiency and consequently animal performance.

1. Introduction

The goal of the ruminant nutritionist is to improve rumen metabolism efficiency and productivity of animals (Patra and Saxena, 2011). Therefore, a wide variety of feed additives such as antibiotics, ionophores, methane inhibitors, and defaunating agents have long been used in ruminant nutrition. However, due to the presence of chemical residues in ruminant-derived food products and antibiotic resistance of bacteria, scientists have become interested in exploring natural alternatives to modulate rumen fermentation and increase eco-friendly animal productions.

Biochar is a porous, solid carbon-rich material produced when biomass, typically trees, forages, straws and agricultural and animal waste is subjected to pyrolysis with a limited amount of oxygen (Lehmann and Joseph, 2009). Biochar production is a new strategy in agricultural waste management in order to minimize environmental pollution and improve soil properties. More recently, biochar has been proposed as a supplement in ruminant diets (Schmidt et al., 2017).

A large biomass of about 560,000 tonnes of pistachio by-product and 65,000 tonnes of walnut shell is produced annually in Iran, which will primarily be burned or discharged into the environment, which can cause detrimental environmental effects (Mirheidari et al., 2018). Poultry litter manure is another source of environmental pollution, which can easily be converted to biochar and used in animal nutrition as a low cost feed additive. Manufacture and use of biochar from these unused resources not only is a key to managing animal and crop wastes directly, but also can mitigate climate change indirectly (Lehmann and Joseph, 2009). In addition, inclusion of biochar in ruminant diets may have benefits such as reducing enteric methane emissions (Leng et al., 2012a), improving animal health and feed efficiency (Schmidt et al.,

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https://doi.org/10.1016/j.smallrumres.2019.106042
Received 21 January 2019; Received in revised form 14 September 2019; Accepted 18 December 2019
Available online 19 December 2019

2017), and excreting dietary biochar as a low cost method of transferring biochar to pasture and soil in order to improve soil fertility (Joseph et al., 2015).

Despite its potential benefits, chemo-physical properties of biochar may vary depending upon the biomass source. We hypothesized that incorporation of biochar in the diets of lambs may improve production performance, and different resources of biochar may have different effects on animal responses. Therefore, the aims of this study were therefore to determine the effect of adding different biochar produced from different biomass sources on feed intake, digestibility, rumen fermentation, microbial protein synthesis, and daily gain in male lambs.

2. Materials and methods

2.1. Biochar preparation

The biochars used in this study were produced by pyrolysis of walnut shell, pistachio by-product, and chicken manure. The biomass was stocked into a smaller barrel as tight as possible, then the whole barrel was inverted into a larger one and the space between the barrels was then filled with wood and ignited. The larger barrel was heated from below with a gas heat source until it reached pyrolysis temperatures (above 550 °C) for 3 h. The biochar was cooled down with water spray and then sun dried (Table 1).

2.2. Animals, diets and experimental design

Twenty four Kermanian ram lambs with an average weight of 21.90 ± 2.24 kg, (3–4 months of age) were allocated to four experimental diets in a completely randomized design (n = 6). Experimental diets contained, as a DM basis, 1 % WSB (walnut shell biochar), 1 % PBB (pistachio by-product biochar), and 1.5 % CMB (chicken manure biochar) (Table 2).

All lambs were housed in individual pens (1.2 m × 1.5 m) with free access to feed and water. The experiment consisted a 14-d adaptation period, followed by a 90-d period during which body weight (BW) changes and dry matter intake (DMI) were recorded. Diets were formulated to be iso-caloric and iso-nitrogenous and were fed twice daily in two equal portions at 07:00 and 19:00 with a 40:60 forage to concentrate ratio. Before the beginning of the experiment, all animals were treated against internal and external parasites and routine vaccination. Lambs were visited weekly in a regular program for general health and individual behaviors (i.e. rumination, illness, heart rate, breath and anorexia). Animal handling and experimental procedures were performed according to the guidelines approved by Iranian Council of Animal Care (1995).

2.3. Nutrient digestibility

Direct fecal samples were collected from rectum five times daily on days 78–82 (11th wk) and then dried in an oven. Daily dried fecal samples were ground and later composited for the 5-day collection period. Diets were sampled daily and composited over the 7-d period. Apparent total tract digestibility was estimated using acid insoluble ash (AIA) as an intrinsic digestibility marker (Van Keulen and Young, 1977).

Table 1
Chemical properties of biochars produced from walnut shell (WSB), pistachio by-product (PBB), and chicken manure (CMB).

Item	Biochar		
	WSB	PBB	CMB
Dry matter	97.3	92.8	96.3
pH	9.19	10.67	9.50
Composition (%)			
C	88.02	57.93	24.27
H	2.38	2.00	0.94
N	0	1.67	1.35
Yield† (%)	72.03	67.74	58.60

† Calculated as (mass of biochar (g) / oven dry mass of feedstock (g)) × 100 (Zhao et al., 2017).

Table 2
Ingredients and chemical composition of experimental diets (DM basis).

Ingredients (%)	Experimental diets†			
	CON	WSB	PBB	CMB
Alfalfa	30	30	30	30
Wheat straw	9.8	9.8	9.8	9.8
Barley grain	49.4	48.6	48.6	48.2
Corn grain	4.0	4.0	4.0	4.0
Soybean meal	3.9	3.7	3.7	3.6
Wheat bran	1.7	1.7	1.7	1.7
Biochar	0	1.0	1.0	1.5
Mineral-vitamin premix‡	1.0	1.0	1.0	1.0
Salt	0.2	0.2	0.2	0.2
Chemical composition (%)				
Metabolizable energy (Mcal/kg)§	2.55	2.52	2.53	2.50
Dry matter	91.5	90.5	90.5	90.1
Organic matter	93.0	92.9	92.3	91.3
Crude protein	14.1	14.1	14.0	14.2
Neutral detergent fiber	32.9	33.4	33.5	33.5
Acid detergent fiber	19.7	20.3	20.3	20.4
Non-fiber carbohydrate¶	44.0	43.4	42.8	41.6

† Experimental diets contained without biochar as a control (CON), walnut shell biochar (WSB), pistachio by-product biochar (PBB), and chicken manure biochar (CMB).

‡ Each kg of the vitamin–mineral premix contained: vitamin A (50,000 IU), vitamin D3 (10,000 IU), vitamin E (1500 IU), Calcium (155 g), Phosphorus (40 g), Sodium (71 g), Magnesium (19 g), Iron (3 g), Copper (1.3 g), Manganese (5.5 g), Zinc (12 g), Cobalt (32 mg), Iodine (55 mg), and Selenium (10 mg).

§ Calcuted from NRC (2007).

¶ Non-fibre carbohydrates calculated as 100 - (NDF + CP + ether extract + ash).

2.4. Urine collection

The complete output of urine was collected daily from each animal into a bucket containing approximately 100 ml of 10 % H_2SO_4 on days 85–90 (12th wk) of the experimental period (Chen and Gomes, 1995). Urine was weighed once a day and mixed well, and a 10 % daily aliquot was pooled over the 5-day collection period for each animal, diluted threefold, and stored at −20 °C for total purine derivatives (i.e. allantoin, uric acid, xanthine plus hypoxanthine) analyses.

2.5. Rumen fluid sampling

Rumen fluid samples were collected from each lamb by a stomach tube 3 h after the morning feeding on day 91. The pH value was measured immediately using a portable pH meter and then strained through two layers of cheesecloth. A subsample of 5 ml rumen fluid was combined with 5 ml of 0.2 N HCl, and another 5 ml subsample was mixed with 1 ml of 25 % meta-phosphoric acid (w/v) for ammonia-N and volatile fatty acid (VFA) analyses, respectively. All samples were stored at −20 °C for further analyses. Two mL of rumen fluid was pipetted into screw-capped test tubes containing an equal volume of 50 % Formalin (18.5 % concentration of formaldehyde) and stored for counting protozoa.

2.6. Laboratory analyses

Dry matter (DM) (method 934.01), ash (method 942.05) and acid

detergent fiber (ADF) (method 973.18) were determined according to AOAC (2005). Crude protein (CP) (method 976.05) was determined by an automatic Kjeldahl analyzer (Kjeldahl Vap50 Gerhardt, Germany) as described in AOAC (2005). Neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991). Both NDF and ADF were expressed exclusive of residual ash. The total content of C, H, and N in biochars were determined using a CHNS analyzer (Thermo Finnigan, Flash EA 1112 Series).

Protozoa were counted according to Dehority (1984). Briefly, 1.0 ml aliquot of the formalinized sample was pipetted into a test tube and then two drops of brilliant green were added, mixed thoroughly and allowed to stand overnight at room temperature. After addition of 9 ml of a 30 % glycerol solution, the sample was pipetted into a counting chamber. Total counts of protozoa were made in 30 microscopic fields at a magnification of × 20 in a haemocytometer (Neubauer improved, Marienfeld, Germany).

The ammonia N concentration of rumen fluid samples was analyzed by the procedure of Weatherburn (1967). Volatile fatty acids were analyzed using a gas chromatograph (YL6100 GC; Young Lin Instrument, Anyang, South Korea) equipped with a 50 mm internal diameter) silica-fused column (CP-Wax Chrompack Capillary Column; Varian, Palo Alto, CA, USA). Helium was used as the carrier gas, and initial and final oven temperatures were set at 55 and 210 °C, respectively. Detector and injector temperatures were set at 250 °C. Crotonic acid (1:7 v/v) was used as the internal standard.

The amounts of allantoin, uric acid, and xanthine plus hypoxanthine were determined by spectrophotometric method (Chen and Gomes, 1995) with a non-linear equation for describing the quantitative relationship between absorption of microbial purines and excretion of purine derivatives (PD) in urine:

$$y = 0.84 \times X + (0.150 \times BW^{0.75} e^{-0.25x})$$

where y is the daily urinary PD excretion in mmol/d, X is the daily absorbed exogenous purines in mmol/d, and $W^{0.75}$ is the metabolic body weight (kg) of the animal. The supply of microbial N (g/day) was estimated as follows:

$$MN = (X \times 70) / (0.116 \times 0.83 \times 1000) = 0.727 \times X$$

where 0.83 is the digestibility of microbial purine, 70 is the N content of purines (mg N/mmol), and 0.116 is the ratio of purine-N: total N in mixed rumen microbes.

2.7. Statistical analysis

All variables were statistically analyzed using the mixed model procedure of SAS Institute Inc. (2003), with animal serving as the experimental unit. The class statement included lamb and treatment and the model statement included treatment. For analyzing data on performance, the initial body weight was introduced as a covariate $b(x\text{-}\bar{x})$ in the model.

Means separation was determined using the least square means (LSMEANS) statement with the PDIFF option, and all means were reported as least squares means. Treatment effects were considered significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

3. Results

3.1. Intake, growth, and digestibility

Data on performance variables are presented in Table 3. DMI was not affected by biochar; however, final BW, average daily gain (ADG), and feed conversion ratio (FCR) improved in lambs fed biochar compared to control ($P < 0.05$).

The highest digestibility coefficients of DM, OM, CP, and NDF were observed in WSB fed lambs; however, no significant difference was detected in lambs supplemented with WSB and those fed CMB in terms of NDF digestibility (Table 3).

Table 3
Least square means comparisons of experimental diets for intake, performance and apparent total tract digestibility in Kermanian male lambs.

Item‡	Experimental diets†				SEM	*P* value
	CON	WSB (1 %)	PBB (1 %)	CMB (1.5 %)		
Body weight (kg)						
Initial	21.42	22.17	21.67	22.33	0.456	0.89
Final	36.58^{b}	45.33^{a}	43.10^{a}	45.41^{a}	0.921	< 0.001
DMI (g/d)	1295	1276	1348	1245	29.4	0.79
ADG (g/d)	164.4^{b}	249.3^{a}	239.4^{a}	247.9^{a}	10.14	0.002
FCR	8.20^{a}	5.68^{b}	5.93^{b}	5.95^{b}	0.328	0.01
Digestibility (%)						
DM	65.47^{b}	80.22^{a}	71.37^{b}	68.65^{b}	1.646	0.004
OM	68.34^{b}	80.96^{a}	72.54^{ab}	67.29^{b}	1.909	0.03
CP	67.76^{b}	80.37^{a}	72.50^{b}	72.64^{b}	1.458	0.01
NDF	62.63^{b}	75.53^{a}	66.24^{b}	72.77^{a}	1.293	< 0.001

[a,b]Least square means within a row with different superscripts differ significantly ($P < 0.05$).

† Experimental diets contained without biochar as a control (CON), walnut shell biochar (1 % WSB), pistachio by-product biochar (1 % PBB), and chicken manure biochar (1.5 % CMB).

‡ DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; DM, dry matter; OM, organic matter; CP, crude protein; and NDF, neutral detergent fiber.

3.2. Ruminal parameters

Ruminal NH_3-N concentration increased with inclusion of WSB and PBB into diets ($P = 0.006$). The pH value, total counts of rumen protozoa, concentrations of total VFA, and molar proportion of individual VFA were not affected by addition of biochar produced from different substrates (Table 4). However, there was a trend for WSB treatment to result in lower acetate to propionate ratio than control ($P = 0.07$).

3.3. Purine derivatives and microbial protein synthesis

The concentration (mmol/d) of allantoin, uric acid, and total PD absorbed and rumen microbial nitrogen synthesis (MNS) (g N/d) were increased with incorporation of all sources of biochar in the diet

Table 4
Least square means comparisons of experimental diets for ruminal fermentation parameters and rumen protozoa in Kermanian male lambs.

Item‡	Experimental diets†				SEM	*P* value
	CON	WSB (1 %)	PBB (1 %)	CMB (1.5 %)		
pH	6.55	6.35	6.53	6.48	0.078	0.83
Ammonia-N (mg/dL)	6.77^{c}	10.34^{a}	9.04^{ab}	8.35^{bc}	0.396	0.006
Rumen protozoa (log10/(cfu/ml))	8.01	11.84	10.67	12.83	0.769	0.13
Total VFA (m*M*)	108.5	113.1	110.3	112.0	1.30	0.43
Individual VFA (mmol/100 mol)						
Acetate	62.83	66.20	63.79	65.51	1.165	0.82
Propionate	20.59	25.56	22.43	21.64	0.903	0.25
Butyrate	12.93	13.82	13.31	13.39	0.203	0.60
Acetate: Propionate	3.06	2.59	2.85	3.04	0.080	0.07

[a,b,c] Least square means within a row with different superscripts differ significantly ($P < 0.05$).

† Experimental diets contained without biochar as a control (CON), walnut shell biochar (1 % WSB), pistachio by-product biochar (1 % PBB), and chicken manure biochar (1.5 % CMB).

‡ VFA; volatile fatty acid.

Table 5
Least square means comparisons of experimental diets for urinary purine derivatives excretion and microbial protein synthesis in Kermanian male lambs.

Item‡	Experimental diets†				SEM	*P* value
	CON	WSB (1 %)	PBB (1 %)	CMB (1.5 %)		
Allantoin (mmol/d)	9.15[b]	13.82[a]	13.75[a]	11.67[a]	0.536	0.001
Uric acid (mmol/d)	1.92	3.32	2.47	3.70	0.296	0.13
X + H (mmol/d)	0.64[b]	1.03[a]	0.93[a]	0.99[a]	0.053	0.03
TPD absorbed (mmol/d)	13.85[b]	21.61[a]	20.39[a]	19.45[a]	0.856	0.002
Microbial N supply (g N/d)	10.07[b]	15.71[a]	14.82[a]	14.14[a]	0.622	0.002
EMNS (g N/kg DOMR)	19.05[b]	25.90[ab]	25.75[ab]	29.34[a]	1.364	0.045

[a,b] Least square means within a row with different superscripts differ significantly ($P < 0.05$).

† Experimental diets contained without biochar as a control (CON), walnut shell biochar (1 % WSB), pistachio by-product biochar (1 % PBB), and chicken manure biochar (1.5 % CMB).

‡ X + H, xanthine + hypoxanthine; TPD, total purine derivatives; EMNS, efficiency of microbial nitrogen synthesis; and DOMR, apparently digested organic matter in the rumen (65 % of apparently digested organic matter in total tract) according to ARC (1984).

($P < 0.05$) (Table 5). Efficiency of microbial nitrogen synthesis (EMNS) (g/kg apparently digested organic matter in the rumen (DOMR)) was higher in CMB fed lambs compared to those fed a basal diet without biochar, but no differences were found between biochar treatments produced from different sources There was also a tendency ($P = 0.07$) for more EMNS in lambs supplemented with WSB or PBB compared to control.

4. Discussion

4.1. Intake, growth performance and digestibility of nutrients

The levels of biochars to be incorporated in the diets were arrived based on an in vitro study. To the best of our knowledge, this study may be the first one conducted in vivo using WSB, PBB and CMB. The feeding experiment clearly confirmed the impact of biochar on improving animal production. Increased BW by supplementing with all sources of biochar could be a response to the greater total digestibility of nutrients and enhanced rumen MNS. Similar DMI across treatments and improvement in production performance as a result of biochar inclusion in our study are consistent with Leng et al. (2012b) who evaluated biochar derived from rice husk for the first time in vivo. They reported that feeding rice husk biochar at 0.6 % of basal diet DM consisting of root chips and fresh cassava foliage in a yellow cattle ration had no effect on DMI, but increased ADG and final BW. Van et al. (2006) reported that goats fed a diet supplemented with bamboo charcoal at 0.5 g/kg of live weight per day grew faster (20.4 %) than those fed the same diet without charcoal.

Since optimizing rumen microbial growth milieu will improve ruminant production (Beever and Drackley, 2013), inclusion of biochar in the diet may create a new microbial habitat in the rumen, which can improve microbial growth efficiency through close and specific association of different microbes species (Leng et al., 2012b). This effect can be explained by the highly porous structure of biochar, which provides more surface area for attachment of microorganisms and biofilm development while delivering beneficial substances such as starch, proteins and plant oils to animals (Belcher et al., 2017). It has been generally accepted that attachment of rumen microbes to feed particles is necessary for initiation of the consortia in order to solubilize and ferment feed OM, and particle associated microbes which are formed in a biofilm matrix play the most important role in rumen digestion (Edwards et al., 2008). Therefore, the biofilm formation greatly increased rate of digestion via syntrophism in microbial communities (Leng, 2014).

Given the fact that all diets were iso-caloric and iso-nitrogenous the greater digestibility in WSB fed lambs may suggest a stimulating effect on activity of microbial communities through its support for biofilms in the digestive tract of the animal and improvement in rumen fermentation because of its higher content of C compared to other sources of biochar. Hang et al. (2018) reported that apparent DM digestibility of goats fed urea treated cassava stems was increased (9 %) by supplementing with rice husk biochar at 1 % of DM intake. In contrast, digestibility of DM, OM, and CP of goats fed Bauhinia foliage as the basal diet was not affected by 1 % biochar supplementation (Silivong and Preston, 2015). Generally, biochar can provide a shelter for growth and propagation of microorganisms and improved the synergism between nutrients and microorganisms in the animal's digestive system (Silivong et al., 2018), thereby enhancing digestibility

4.2. Ruminal parameters

The stability of ruminal pH among treatments can be attributed to the lack of change in the primary ruminal fluid VFA, acetate and propionate concentrations (Table 4). This result is in contrast to those obtained by Cabeza et al. (2018), who reported a reduction in ruminal pH when biochar prepared from Miscanthus straw, oil seed rape straw and soft wood pellets at 1.16 % of feed substrate were added to incubations as though these differences were small and ranged from 6.51 to 6.55. On the other hand, feeding rice husk biochar at 0.6 % of diet DM to yellow cattle increased pH of rumen fluid (Leng et al., 2012b). These differences may be due to various diet ingredients and the type of biochar used.

In our study, inclusion of WSB, PBB, and CMB increased ruminal NH_3-N concentration by 34.5 %, 25.06 %, and 18.89 %, respectively, which is consistent with Leng et al. (2012b) who observed that addition of 0.6 % biochar significantly increased ruminal ammonia concentration in Yellow cattle. However, 0.3 % activated carbon in concentrated based diets decreased the concentration of ruminal ammonia-N in female Suffolk sheep before feeding (Garillo et al., 1995). In vitro studies showed no effect (Calvelo Pereira et al., 2014) or a reduction in concentration of ammonia by supplementing with different material sources of biochar (Cabeza et al., 2018). This reduction was most marked in biochar derived from Miscanthus straw, which might be attributed to a reduction in proteolysis and deamination and/or higher incorporation of NH_3-N to microbial protein (Cabeza et al., 2018). Higher ruminal ammonia-N in our study may be partially due to a numerically increased in protozoal population (Table 4) (Doreau and Ferlay, 1995). Another possibility can be a greater protein digestion in lambs fed biochar treatments, especially WSB, as protein is extensively degraded in the rumen (Tamminga, 1979).

The amount of total VFA production was not influenced by biochars. Similarly McFarlane et al. (2017) did not observe any changes in VFA production with addition of biochar in vitro. Cabeza et al. (2018) reported that the concentrations of total VFA or acetate were not affected by addition of 1.16 and 11.6 % of substrate DM biochar produced from different biomass sources to incubations; however, proportions of propionate and butyrate were reduced by inclusion of biochar. In contrast, Calvelo Pereira et al. (2014) found that total VFA production and acetate concentration increased as the level of biochar in the grass silage increased from 8.11–18.6% of DM, whereas the amount of propionate was decreased and butyrate was not affected by the level of biochar. They also stated that the type of biochar (pine wood chips or corn stover) had no effect on total VFA and molar proportion of individual VFA either when biochar was mixed with hay (at 16 % of DM) or silage (8.11 and 18.6 % of DM). Direct addition of pine biochar to hay or grass silage previously mixed with biochar at 18.6 % DM lowered acetate proportion compared to grass, which was attributed to the difference between the grass and the silage (Calvelo Pereira et al.,

2014).

4.3. Urinary excretion of purine derivatives and microbial nitrogen supply

Regarding similar DM and nitrogen intakes, higher amounts of TPD and MNS in biochar fed lambs compared to control group can be due to higher ruminal NH_3-N, which is a principal source of N for microbial protein synthesis and growth performance (McDonald et al., 2011). Moreover, an increase in colonizable surfaces as a result of biochar addition may lead to an increase in microbial growth and biomass. Direct comparison with other data is not possible due to lack of literature on the effect of biochar on microbial protein synthesis. However, Leng et al. (2012b) reported that addition of biochar increased microbial growth efficiency in vivo. It has been stated that the low redox potential in the rumen is essential for microbial activity and dynamics of fermentation (Marden et al., 2005). Biochar, as a reducing agent, may maintain a stable environment in the rumen, hence improving microbial activity, but as no direct measurements of redox potential were made, then this is speculative. Rumen microbial protein is an important contributor to metabolizable protein in ruminants and consequently for maintenance and production (Ørskov, 1982) and provides the majority of protein supplied to the small intestine of ruminants, accounting for 50–80% of total absorbable protein (Storm and Ørskov, 1983). Thus, application of biochar in the diets of ruminants can be an alternative strategy for enhancing microbial protein synthesis.

The values of EMNS obtained in the present study (19.05–29.34 g/kg DOMR) were within the range of 14 – 49 g/kg DOMR specified by ARC (1984). A numerically higher efficiency of MNS in lambs fed CMB compared to those fed WSB or PBB could be a consequence of lower OM digestibility.

5. Conclusion

Our results indicated that addition of WSB, PBB, and CMB to the diet dramatically increased growth performance and MNS. Feed conversion ratio was also improved with biochar supplementation. However, with the exception of increased DM, OM and CP digetibilities, no significant advantage of adding WSB compared with supplementing with other sources of biochar (PPB and CMB) was observed. In conclusion, positive effects of biochar suggests that, as a low cost feed additive, biochar can be beneficially incorporated in fattening sheep diets to increase MNS and ultimately productivity of animals.

Declaration of Competing Interest

We wish to confirm that there is no conflict of interest associated with this publication.

Acknowledgements

This work was not funded by any organization. The authors would like to acknowledge Mr. H. Mirheidari and Mr. K. Mirheidari for their assistance in various aspects of the experiment.

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