

Effect of Biochar Type and Size on *in Vitro* Rumen Fermentation of Orchard Grass Hay

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Abstract

Biochar, a byproduct from the biofuels industry, may be a potential feed additive in ruminant diets due to possible improvements in microbial fermentation. Therefore, the objective of this study was to determine the nutritive value, *in vitro* digestibility, volatile fatty acid (VFA) production, and gas production of biochar inclusion to an orchard grass (*Dactylis glomerata*) basal diet. The study was designed as a 3 × 2 factorial arrangement with 3 different biochar sources and 2 biochar processed sizes as the main effects factors. Experimental treatments were biochar from 3 different tree types: 1) Chestnut Oak (*Quercus prinus* L.; CO), 2) Yellow Poplar (*Liriodendron tulipifera*; YP), or 3) White Pine (*Pinus strobus* L.; WP), and processed at 2 different biochar particle sizes: a) <178 μm (Fine) or b) >178 μm (Coarse). Biochar was added to the basal diet of orchard grass hay (872.35 g/kg of DM, 98.31 g/kg of CP, and 704.02 g/kg of aNDF, DM basis) at a rate of 81 g/kg DM. Biochar residual ash content was greater ($P < 0.01$) for Fine particle size and greater ($P < 0.01$) for CO and YP biochar sources. Biochar aNDF content exhibited a type × size interaction ($P = 0.01$) with lower aNDF content in both WP sizes compared with their respective biochar type and size. Gas production was not influenced ($P = 0.23$) by biochar tree type; however, gas production was increased ($P = 0.05$) by Fine particle size compared with Coarse biochar. The *in vitro* true digestibility (IVTD) of orchard grass hay was increased ($P = 0.01$) by the inclusion of Fine biochar particle size compared with Coarse particle size. Additionally, *in vitro* CP true digestibility (DCP) exhibited a type × size interaction ($P = 0.01$). Crude protein digestibility was lower for Fine particle-sized CO and WP biochar sources compared with Coarse particle-sized CO and WP ($P \leq 0.004$). However, DCP was not different between Coarse and Fine particle-sized YP biochar ($P = 0.70$). Volatile fatty acids (acetate, propionate, and butyrate) were not altered by biochar type ($P \geq 0.66$) or particle size ($P \geq 0.19$). These results indicate that both tree type and particle size of biochar may need

to be carefully considered before incorporating into a ruminant diet. Furthermore, Fine particle-sized biochar may be the most effective to incorporate as a feed additive in a ruminant diet based on digestibility parameters.

Keywords

Biochar, Forage Digestibility, Gas Production, Rumen Fermentation

1. Introduction

Biochar is a byproduct attained when biomass is subjected to pyrolysis, typically in the biofuel industry. This process subjects biomass, such as trees, forages, straws, and agricultural waste, to heat at moderately low temperatures with a limited amount of oxygen [1]. Biochar and charcoal are produced similarly, which activated charcoal has been used as a feed additive in ruminant diets [2] [3]. In addition, charcoal has been used as an additive to improve growth performance, feed efficiency, and as a detoxifying agent in ruminants [4]. Comparatively to charcoal, the increased surface area and porous structure of biochar may aid in gas sorption, providing the potential as a feed additive [1]. Research focusing on methane mitigation have shown promise with biochar during *in vitro* [5] [6] [7] and *in vivo* [8] studies. However, more extensive research is necessary to determine the potential benefits and impacts of biochar on forage digestibility and rumen fermentation kinetics. Thus, our hypothesis was that biochar type would not impact rumen fermentation, but a larger particle size would inhibit rumen fermentation. Therefore, the objective of this study was to determine the effect of biochar source and particle size on nutritive value, *in vitro* forage digestibility, volatile fatty acid (VFA) production, and gas production.

2. Materials and Methods

2.1. Study Design

Treatments were arranged as a 3 × 2 factorial with 3 commercial biochar tree types: 1) yellow poplar (*Liriodendron tulipifera*; YP), 2) white pine (*Pinus strobus* L.; WP), or 3) chestnut oak (*Quercus prinus* L.; CO) and 2 different biochar particle sizes: a) <178 μm (Fine) or b) >178 μm (Coarse). The basal diet (872.35 g/kg of DM, 98.31 g/kg of CP, and 704.02 g/kg of aNDF, DM basis) consisted of orchard grass (*Dactylis glomerata*) hay. Biochar was added to the basal diet at a rate of 81 g/kg of DM.

2.2. Biochar Production

Biochar was provided by Proton Power, Inc., Lenoir City, TN. Proton Power utilized tree types that are highly present in Eastern Tennessee. For each tree type, wood chips were generated separately from whole logs using a brush chipper (Vermeer BC1000XL, Pella, IA). Wet wood crumbles were generated by using a Forest Concepts 6.4 mm M24ci Crumbler (Forest Concepts, LLC, Auburn, WA)

and then were pyrolyzed using a Proton Power CHyP engine (Proton Power, Inc., Lenoir City, TN) at a peak temperature of 1110°C with a residence time greater than 3 min. Biochar was collected in a sealed metal bin and allowed to cool. Biochar samples were fractionated utilizing a single deck orbital separator (SWECO LS24S44, SWECO, Florence, KY) equipped with a Market Grade 80 mesh screen (178 µm opening) woven with a 0.14 mm diameter stainless steel wire. For each tree species, one sample (Fine) was collected from the sieved biochar particles below the 80 mesh screen (178 µm opening) and one sample (Coarse) was collected from the biochar particles remaining on top of the screen.

2.3. Scanning Electron Microscopy

The different biochar types were characterized by scanning electron microscopy (SEM) in a FEI Quanta 200 at 15 kV. Secondary electron (SE) imaging revealed topographical information, and back-scattered electron (BSE) imaging modes emphasized features due to atomic number contrast. Biochars examined were affixed to conventional specimen stubs via the use of double-sided adhesive tape; the adhesive surface was merely pressed into the sample of char, placed into the SEM, and imaged.

2.4. Forage Proximate Analysis

Orchardgrass hay and biochar samples were analyzed for dry matter (DM), ash, crude protein (CP), and neutral detergent fiber (aNDF) content. The DM content of the samples was determined by drying at 55°C in a forced-air oven for 48 h. Samples were then lyophilized and ground through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Crude protein was determined by total N combustion analysis (Leco Instruments, Inc., St. Joseph, MI) [9]. Ash was determined after complete combustion in a muffled furnace (L&L Special Furnace Co., Inc., XLB Series, Aston, PA) at 550°C for 6 h [9]. Neutral detergent fiber (aNDF) content was assessed utilizing the ANKOM 200 fiber analysis system (ANKOM Corp., Fairport, NY).

2.5. Gas Production Sample Preparation

To determine 48 h gas production, rumen extrusa was immediately added to Ankom^{RF} Gas Production system modules (ANKOM Corp., Fairport, NY) along with the basal diet (0.50 ± 0.01 g) and biochar at a level of 81 g/kg DM as previously reported by [10]. Rumen fluid was collected into a preheated thermal flask (39°C ± 0.5°C) and filtered through 3 layers of cheesecloth to eliminate feed particles. Rumen fluid (35 mL) was buffered utilizing McDougall's artificial saliva [11] in a 1:4 dilution of rumen fluid to buffer. Each bottle (620 mL) was filled with rumen extrusa and sample leaving a corresponding headspace volume of 585 mL. The bottles were placed into a water bath (Orbital Water Bath Shaker, Model 3540, Lab Line, Melrose Park, IL) at 39°C ± 0.5°C for 48 h while being agitated (50 RPM). Data was automatically generated by the Ankom pressure sensor module (pressure accuracy: 0 ± 0.2 psi accuracy) to a local computer at

intervals of 5 min. Gas production data were analyzed to compare the changes in pressure in the head space at 0, 3, 6, 12, 24, and 48 h of incubation in triplicates.

2.6. VFA Analysis

An analytical subsample of rumen fluid was collected from each Ankom^{RF} Gas Production system module (ANKOM Corp., Fairport, NY) at the end of each 48 h incubation experiment for VFA analysis. Rumen samples were prepared by centrifuging strained samples at 10,000 ×g for 10 min at 4°C. A mixture of 5 mL of ruminal fluid supernatant and 1 mL of meta-phosphoric acid-2ethyl butyric acid solution was then prepared. This mixture was allowed to stand in an ice bath for ≥30 min and then prepared for a second centrifuge for 10 min at 10,000 ×g and 4°C. The samples were then analyzed using a gas chromatograph (Agilent 7890B, Agilent Technologies, Inc., Santa Clara, CA) with the previously described method [12]. The gas chromatograph was equipped with a FID detector, Nukol Fused Silica capillary column (Supelco, Sigma-Aldrich Co., LLC, Bellefonte, PA), and Helium as the carrier gas.

2.7. Forage Digestibility

The Daisy II *in vitro* fermentation system (ANKOM Corp., Fairport, NY) was utilized to examine the rate and extent of DM and fiber degradation of forages with the addition of biochar. Each substrate (500 ± 20 mg) was weighed into artificial fiber bags (#F57, ANKOM Corp.), which were then heat-sealed. Groups of 24 bags, including two empty bags for correction, were placed upright in plastic containers. Rumen fluid was buffered utilizing a 1:4 dilution of rumen fluid to buffer. Ruminal fluid (400 mL) was added to each ANKOM fermentation jar with an additional 1600 mL of buffer [13] that was adjusted to pH 6.8. Bags, in addition to all liquid contents in the plastic containers, were then added to the fermentation jars. Fermentation was initiated for 48 h at 39°C. Bags were removed and washed under cold tap water until excess water ran clear. Fiber (aNDF) degradation was determined sequentially on the same bags with α -amylase and sodium sulfite (Mertens, 2002) using the ANKOM200 fiber analysis system (ANKOM Corp., Fairport, NY). Crude protein was determined by total N combustion analysis (Leco Instruments, Inc., St. Joseph, MI).

2.8. Statistical Analysis

Samples were analyzed as a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC, USA) to test differences among the 7 treatment combinations, and contrasts (without control) tested main effects of biochar type and size and their interaction. Repeated measures were utilized for variables collected over time. Least squares means were compared using Fisher's LSD at a significance level of $P \leq 0.05$.

3. Results

Dry matter was not different among biochar tree types ($P = 0.22$; **Table 1**) or

Table 1. Chemical composition of different biochar tree types (CO, Chestnut Oak; YP, Yellow Poplar; WP, White Pine) and particle sizes.

| Measurements | Treatment | | | | | | SEM | Type | P-value | |
|--------------|---------------------|----------------------|---------------------|----------------------|---------------------|---------------------|--------|-------|---------|---------------|
| | CO | | YP | | WP | | | | Size | Source × Size |
| DM (g/kg) | 976.82 ^a | 954.37 ^a | 960.23 ^a | 982.32 ^a | 856.48 ^a | 952.12 ^a | 372.18 | 0.22 | 0.34 | 0.34 |
| Ash (g/kg) | 187.86 ^a | 72.41 ^{bc} | 199.37 ^a | 71.74 ^{bc} | 95.35 ^b | 44.62 ^c | 14.44 | 0.007 | 0.001 | 0.08 |
| CP (g/kg) | 21.21 ^{ab} | 19.94 ^{ab} | 19.90 ^{ab} | 24.71 ^a | 10.98 ^b | 18.48 ^{ab} | 3.22 | 0.12 | 0.21 | 0.43 |
| aNDF (g/kg) | 800.71 ^c | 1002.48 ^a | 843.79 ^c | 1027.09 ^a | 666.31 ^d | 954.07 ^b | 12.45 | 0.001 | <0.001 | 0.01 |

^{abc}Means within a row, and within a category, with different superscript letters differ ($P < 0.05$). aNDF, Neutral Detergent Fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CO, Chestnut Oak; Coarse, 300 - 1000 μm ; CP, crude protein; DM, dry matter; Fine, 140 - 178 μm ; SEM, standard error of the mean; YP, Yellow Poplar; WP, White Pine.

between particle sizes ($P = 0.34$). In addition, CP was not different for tree type ($P = 0.12$) or particle size ($P = 0.21$) of biochar. Residual ash content of the CO and YP samples was greater ($P = 0.007$) compared to WP biochar. Fine particle-sized biochar had greater ($P = 0.001$) residual ash compared with the larger particle size. In addition, aNDF content exhibited a type \times size ($P = 0.01$) interaction. Neutral detergent fiber was greater for coarse particle-sized biochar for all biochar tree types. Within particle size, there were no differences in aNDF content between CO and YP biochar sources. However, aNDF content was lower for WP biochar than CO and YP biochar sources within their respective particle sizes.

Gas production was not influenced ($P = 0.23$; **Table 2**) by tree type. However, gas production was greater ($P = 0.05$) for fine particle-sized biochar compared to its larger counterpart.

Forage digestibility was not different ($P = 0.35$; **Table 2**) by biochar tree type. However, forage digestibility (IVTD) was greater ($P = 0.01$) with fine particle size compared to coarse. *In vitro* CP digestibility (DCP) exhibited a type \times size interaction ($P = 0.01$). Crude protein digestibility was decreased for fine particle-sized CO and WP biochar sources compared to their larger counterparts. However, DCP was not different between coarse and fine particle-sized YP biochar.

Acetate production was not impacted by tree type ($P = 0.95$; **Table 3**) or particle size ($P = 0.33$). In addition, the production of propionate was not influenced by type ($P = 0.66$) or particle size ($P = 0.19$). Finally, butyrate production did not differ by tree type ($P = 0.97$) or particle size ($P = 0.23$). Acetate: propionate ratio was also not different for biochar type ($P = 0.31$) or particle size ($P = 0.99$).

4. Discussion

Tree type and particle size of biochar samples utilized in this study differed in ash and aNDF content, but did not differ in CP content. The differences in ash and aNDF content may have decreased digestibility of the orchard grass hay

Table 2. Effects of biochar addition on in vitro forage degradability and gas production.

| Measurement ¹ | Treatment | | | | | | SEM | Type | P-value | |
|--------------------------|---------------------|---------------------|---------------------|--------------------|--------------------|---------------------|------|------|---------|---------------|
| | CO | | YP | | WP | | | | Size | Source × Size |
| GP (mL/g DM) | -0.2 ^c | 1.8 ^{abc} | 6.2 ^{ab} | 1.2 ^c | 6.9 ^a | 1.1 ^{bc} | 1.45 | 0.23 | 0.05 | 0.06 |
| IVTD (g/kg DM) | -41.4 ^{ab} | -41.6 ^{ab} | -35.4 ^{ab} | -85.5 ^c | -18.6 ^a | -72.3 ^{bc} | 12.8 | 0.35 | 0.01 | 0.13 |
| DCP (g/kg DM) | -47.7 ^b | 14.5 ^a | -6.9 ^a | -12.7 ^a | -84.2 ^c | -10.1 ^a | 10.3 | 0.02 | 0.001 | 0.01 |

¹These values are representative of the change in response compared to the orchard grass hay control. ^{abc}Means within a row, and within a category, with different superscript letters differ ($P < 0.05$). aNDF, Neutral Detergent Fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CO, Chestnut Oak; Coarse, 300 - 1000 μm ; CP, crude protein; DM, dry matter; Fine, 140 - 178 μm ; SEM, standard error of the mean; YP, Yellow Poplar; WP, White Pine.

Table 3. Effects of biochar addition on in vitro volatile fatty acid production.

| Total VFA (mM) ¹ | Treatment | | | | | | SEM | Type | P-value | |
|-----------------------------|--------------------|--------------------|--------------------|--------------------|-------------------|--------------------|-------|------|---------|-------------|
| | CO | | YP | | WP | | | | Size | Type × Size |
| Acetate | 6.28 ^a | -8.02 ^a | 4.25 ^a | -1.85 ^a | 6.17 ^a | -0.85 ^a | 10.98 | 0.95 | 0.33 | 0.92 |
| Propionate | 1.37 ^a | -2.25 ^a | 1.71 ^a | 0.48 ^a | 0.98 ^a | 0.20 ^a | 1.69 | 0.66 | 0.19 | 0.67 |
| Butyrate | 0.37 ^a | -0.57 ^a | 0.43 ^a | -0.49 ^a | 0.25 ^a | -0.07 ^a | 0.71 | 0.97 | 0.23 | 0.88 |
| Acetate: Propionate | -0.01 ^a | 0.41 ^a | -0.51 ^a | -0.42 ^a | 0.10 ^a | -0.41 ^a | 0.42 | 0.31 | 0.99 | 0.54 |

¹These values are representative of the change in response compared to the orchard grass hay control. ^{abc}Means within a row, and within a category, with different superscript letters differ ($P < 0.05$). aNDF, Neutral Detergent Fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CO, Chestnut Oak; Coarse, 300 - 1000 μm ; CP, crude protein; DM, dry matter; Fine, 140 - 178 μm ; SEM, standard error of the mean; YP, Yellow Poplar; WP, White Pine.

basal diet. Digestibility of forage was likely not impacted by CP content of biochar. Inherent differences among tree types may play a role in the chemical composition, structure, and porosity of the pyrolyzed biochar product (Figure 1). In addition, there may be mineral deposits in the different tree types used for biomass in our study that could be attributed to nutrient cycling of trees during growth or harvest [14]. Biochar is composed of a variety of biomass feedstocks and possesses variable properties due to differences in production and post-production practices [15] [16]. Therefore, biochar characteristics can vary depending on biomass source, region, and pyrolysis procedure which could lead to unavoidable differences in rumen fermentation.

Total gas production increased with the addition of biochar to the basal diet of orchard grass hay. Gas production has been utilized for feed evaluation to assess the kinetics of fiber digestion *in vitro* [17] [18]. Thus, an increase in gas production with the finer particle-sized biochar may indicate an increase in rumen fermentation kinetics. Biochar has been utilized as a feed additive in an attempt to establish a new microbial habitat and may alter biofilm activity in the rumen [19]. In addition, biochar may improve microbial-growth efficiency [6] [8]. However, very little is known how biochar impacts cell wall degradation mechanistically [20]. Previous studies have shown no differences in gas production when biochar is utilized during *in vitro* rumen fermentation [11] [20]. However,

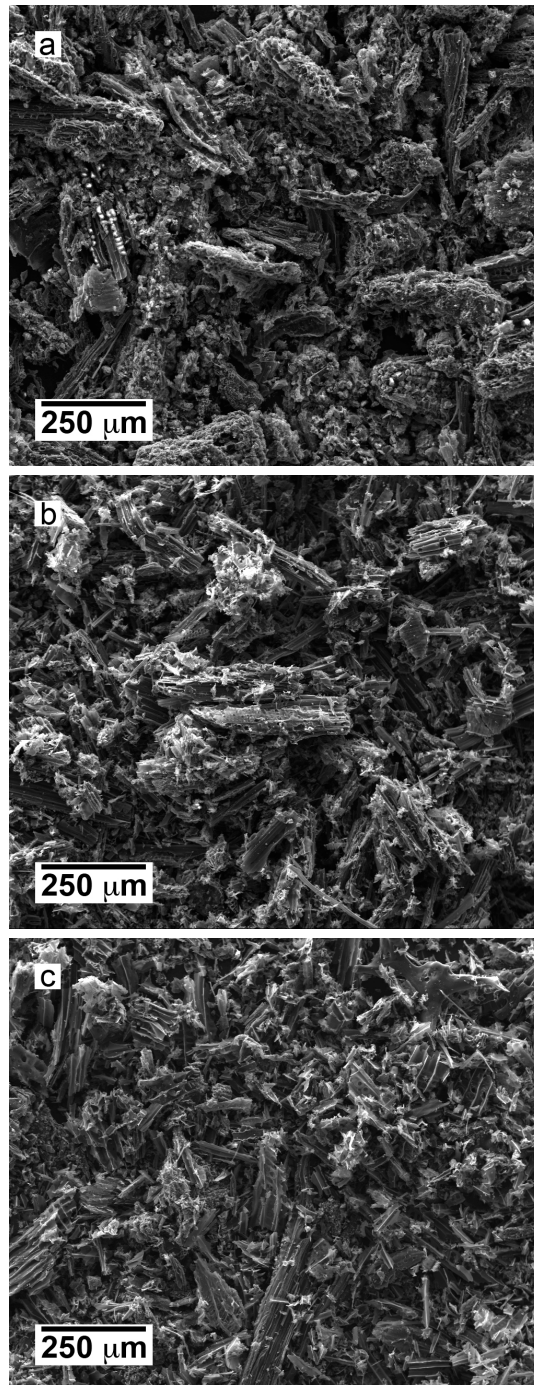


Figure 1. Representative SEM images acquired from Fine (<178 μm) particle size samples of biochars sourced from chestnut oak (a), yellow poplar (b), and white pine (c). Images were acquired at 100×.

biochar utilized in the mentioned studies were composed of various starting materials and biochar particle sizes. Therefore, different sources of biochar may respond differently to rumen fermentation due to structural and compositional differences of the starting material. Additionally, the basal diet utilized in [20] (130 g/kg of CP and 465 g/kg of NDF, DM basis) [11] (104.2 g/kg of CP and 613

g/kg of aNDF, DM basis) are of greater forage quality than the orchard grass hay (98.31 g/kg of CP and 704.02 g/kg of aNDF, DM basis) used in the current study. Thus, biochar may improve rumen fermentation of more moderate-quality forages compared with higher-quality forages.

Biochar decreased *in vitro* true digestibility of orchard grass hay in this study. Biochar source did not influence these differences. However, Fine particle-sized biochar was more digestible. In addition, the digestibility of CP suffered due to Fine particle-sized biochar composed of WP and CO. Thus, biochar source and particle size may differentiate the overall structure and porosity of biochar material potentially impacting microbial forage interaction. Starting material and temperature of pyrolysis can contribute to sorptive potential of biochar [1]. Studies determining the influence of biochar on forage digestibility are limited. However, biochar did not affect DM degradability of a hay and mixed ration substrate [20]. This study utilized biochar from three different sources: gasified biochar, straw-based biochar, and a wood-based biochar as the additives to the diet [20]. Contrarily, biochar produced from rice husks that were subjected to pyrolysis (~900°C) increased the solubilized DM percentage [5]. Ultimately, biochar starting material and particle size may be an important factor to discern the effectiveness of biochar as a feed additive due to changes in forage digestibility.

Volatile fatty acid (VFA) production was not influenced by biochar composition or particle size in this study. In addition, the acetate: propionate ratio was not influenced by biochar addition. In contrast, biochar mixed with grass silage or hay increased VFA production *in vitro* [11]. These differences in VFA production may be attributed to the differing levels of biochar utilized (84 g/kg DM or 186 g/kg DM) and the differences in pyrolysis temperature (350°C or 550°C).

5. Conclusion

Ultimately, biochar may reduce digestibility of forage. Differences in biochar tree type and particle size contributed to differences in gas production and DM degradability in this study. Thus, source and particle size of biochar may be an important consideration when assessing the viability of utilizing biochar as a potential feed additive in ruminant diets. Fine particle-sized biochar may be the most efficacious when incorporating in a ruminant diet due to an increase in gas production and an increase in forage digestibility compared to the larger particle size.

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