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Biochar from pyrolyzed Tibetan Yak dung as a novel additive in ensiling sweet sorghum: an alternate to the hazardous use of yak dung as a fuel in the home

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Abstract

Yak dung is used as fuel in Tibetan homes; however, this use is hazardous to health. An alternative use of the dung that would be profitable and offset the loss as a fuel would be very beneficial. Sweet sorghum silage with yak dung biochar as an additive was compared with a control silage with no additives and three silages with different commercial additives, namely *Lactobacillus buchneri*, *Lactobacillus plantarum* and *Acremonium* cellulase. Biochar-treated silage had a significantly greater concentration of water-soluble carbohydrates than the other silages (76 vs 12.4~45.8 g/kg DM) and a greater crude protein content (75.5 vs 61.4 g/kg DM), lactic acid concentration (40.7 vs 27.7 g/kg DM) and gross energy yield (17.8 vs 17.4 MJ/kg) than the control silage. Biochar-treated and control silages did not differ in *in vitro* digestibility and in total gas (507 vs 511 L/kg DM) and methane production (57.9 vs 57.1 L/kg DM). Biochar inhibited degradation of protein and water-soluble carbohydrates and enhanced lactic acid production, which improved storability of feed. It was concluded that yak dung biochar is an efficient, cost-effective ensiling additive. The profit could offset the loss of dung as fuel and improve the health of Tibetan people.

Keywords: Yak dung biochar; Silage agent; *In vitro* fermentation; Methane emission

1. Introduction

Animal dung is commonly used for fuel in many developing areas (Habtezion, 2013). This is especially true for Tibetan herders, where a reported 12.6 million yaks graze extensively on the natural grasslands of the Qinghai-Tibetan Plateau (Wiener, 2011) and excrete close to an estimated 800 kg of dry dung per yak per year (Degen et al., 2019). Most Tibetan families use only yak dung for cooking and heating (Figure 1a), as they are unable to purchase fossil fuel because of the relatively high costs. However, the burning of yak dung is hazardous to the health of the Tibetans. Due to the long hours of heating (Chen et al., 2011) and the absence of a chimney for most stoves, smoke fills the tents and homes during the combustion of the dung

(Figure 1b), resulting in severe indoor air pollution (Holthaus, 2015; Watts, 2015). Fine particulate matter in these homes was measured at 956 $\mu g/m^3$, whereas the recommended concentration by the WHO Air Quality Guidelines at the time was 25 $\mu g/m^3$ (Xiao et al., 2015). Consequently, the incidences of respiratory disorders, cancer and cardiovascular diseases are high in these Tibetan homes (Pope and Dockery, 2006; Hothaus, 2015), especially in women, as they spend much time near the burning dung. The damage created by the annual 0.4 to 1.7 Gg of black carbon emitted by the combustion of yak dung (Xiao et al., 2015) is substantial, and, today, it is considered a primary cause of global warming (Menon et al., 2002; 2010).

An alternative use of the dung on the Qinghai-Tibetan plateau that would offset the loss of the dung as fuel while being profitable and beneficial for the health of Tibetan herders is needed. In a previous study, a novel, cost-effective biochar from yak dung was developed (Rafiq et al., 2017) (Figure 1c). Biochar has a number of uses, including soil amendment, food conservation and environmental and engineering applications (Farrell et al., 2013). The efficiency of biochar in improving soil properties is dependent on the organic coating, rather than on surface oxidation (Hagemann et al., 2017). When used as a ruminant feed additive, biochar improves nutrient digestibility and animal performance (Mirheidari et al., 2020), while it reduces the uptake of toxicants (Villalba et al., 2002) and the emission of methane (Toth et al., 2016). Hence, integrating biochar in animal feed can be an innovative, beneficial strategy, as biochar absorbs nutrient from the ruminant gut and, subsequently, the feces with the biochar improves soil fertility and grassland productivity (Joseph et al., 2015). Besides these uses, biochar is currently being examined in a number of other fields (Ok et al., 2015) including energy/gas storage, medicinal applications, catalysis, supercapacitors and gas adsorbents. Most of these are still at the initial stage of development (Igalavithana et al., 2018).

Silage is an efficient method in storing feedstock used for biofuel production from energy crops, and is also effective for storing feeds for livestock, in particular to cover periods of feed shortages. Silage can be especially crucial for herders on the Tibetan Plateau during the cold season, when the natural forage is sparse and of poor quality. Sweet sorghum (*Sorghum bicolor*) has garnered much attention as a source of fodder for ruminants, as more than 40% of the dry matter consists of readily fermentable sugars (Henk and Linden, 1992). It produces higher biomass yields while requiring less water and fertilizer than does maize (Qu et al., 2014). Consequently, sorghum has become an important forage and energy crop worldwide, especially in dry areas, and is used widely for silage in China (Xie and Xu, 2019; MOA, 2006).

However, there are challenges in ensiling sorghum due to its coarse structure and high fiber content. Therefore, commercial additives are often used to enhance fermentation and aerobic stability while minimizing the growth of undesirable microorganisms (Pedroso et al., 2010). Many types of microbial inoculants are available on the market. These inoculants are composed mainly of the facultative hetero-fermentative bacterium *Lactobacillus plantarum*, which enhances silage fermentation by lactic acid production and, consequently, rapid reduction in pH (Zhao et al., 2018). In addition, *Lactobacillus buchneri*, which ferments lactic acid to 1,2 propanediol and acetic acid, helps to improve aerobic stability (Oude Elferink et al., 2001). With the growing consumer awareness, probiotic potential of *Lactobacillus sp.* has become the focus of active research. The addition of the enzyme cellulase improves fiber degradation and increases neutral detergent fiber digestibility (Xing et al., 2009). However, the high cost of commercial additives has limited their widespread application. The development of a low-cost, locally produced additive would be of importance to many livestock producers.

Biochar usually has well-developed pore structures, surface functional groups, high stability (Igalavithana et al., 2018) and also provides a surface to support the adherence, growth and catalytic activity of biofilms (Sanchez-Monedero et al., 2018). Biofilm improves the resistance of silage to inhibitory compounds and stimulates microbial action (Lü et al., 2016), while it also strengthens biochar-water interactions and increases nutrient retention (Hagemann et al., 2017; Chen et al., 2020). Furthermore, biochar can enhance hydrogen or electron transfer between methanogens and syntrophic bacteria (Jang et al., 2018), which can reduce enteric CH_4 emission when added to diets of ruminants. Sanchez-Monedero et al. (2018) reviewed the main benefits of biochar in composting, with special attention to greenhouse gas emissions and reduction of nutrient losses. The retention of nutrients is of particular importance in the production of silage (Hagemann et al., 2017). Hence, it was hypothesized that: 1) these beneficial characteristics of biochar could be exploited to improve the nutritional quality of silage forage; and, 2) that dung biochar would prove to be a cost-effective silage additive. To test these hypotheses, the effect of yak dung biochar was examined as an additive in sweet sorghum forage ensiling and compared with three commercial additives. In addition, total gases and methane were determined in an *in vitro* system with rumen fluid, as they are produced in enteric fermentation. Greenhouse gases, in particular methane, has become a worldwide concern and there is reason to believe that biochar can mitigate methane production (Toth et al., 2016). Biochar as an additive in silage fermentation has not been reported elsewhere and, therefore, this study identified a new and previously unexplored area of research. The application of biochar has the potential to have a significant impact on livestock production, especially for farmers in small-scale, rural farming practices who do not have access to or cannot afford current commercial ensiling additives. In this study, sweet sorghum was used for ensiling as it is readily available in China; however, results from this study could be applied to other forages as well.

2. Materials and Methods

2.1 Biochar production and properties

Yak dung was collected manually from a pasture in Maqin County (altitude is 3700 m a.s.l.), Qinghai Province, China. The dung was oven-dried at 65°C, ground into powder (mesh size 100) and pyrolyzed to biochar in a muffle furnace. The dung powder (100 g) was heated at 400°C or 500°C for two hours at a heating rate of 20°C min^{-1} under oxygen limited conditions in a muffle furnace (STM-8-12, Sante, Co, Ltd, Henan, China) (Figure 1c). Slow pyrolysis was used as this produces the most biochar (Monyà, 2012); whereas, fast pyrolysis produces the most bio-oil and gas (Mohan et al., 2014). The biochar sample was passed through a sieve of < 0.15 mm prior to analyses. The physico-chemical characteristics of the biochar were determined earlier (Rafiq et al., 2017; Igalavithana et al., 2018; Table 1). Scanning electron microscopy (SEM) of yak dung biochar used a Zeiss Sigma SEM (Munich, Germany) with a Bruker energy dispersive x-ray analyzer (EDS) as described by Joseph et al. (2015). To provide micro-structural details, scanning transmission electron microscopy (STEM) with electron energy loss spectrometry (EELS) measurements on the C and N K-edges in the porous layer identified carbon and nitrogen functional groups (Mitchell, 2015). In this study, pyrolysis was used to produce biochar as the process is relatively simple and can be adapted by the local population. Hydrothermal liquefaction has been described as an effective and relatively cheap process to produce hydrochar (Cao et al., 2017; 2019). However, this process has a number of limitations including "The requirements of high temperature and pressure that involve the need for highly advanced equipment for use in the reaction process" (Cao et al., 2017).

2.2 Ensiling experiment

Sweet sorghum (*Sorghum bicolor* cv. BMR) was cultivated by the Minshen Forage Production Company (Gansu Province, China), and the silage was prepared at Lanzhou University, Gansu Province, China, from October 2016 to January 2017. The sorghum crop was planted in an area of 20 × 20 m (latitude 38°13′ N, longitude 102°08′ E, altitude 1884 m a.s.l.) from May to September 2016. Sorghum, at a height of 200 cm, was harvested by hand-sickle at the milky growth stage at 15 cm above ground level, pooled and laid on a concrete pad to wilt, and then was chopped to a size of 1 to 2 cm with a lawn mower.

The temperature of 500°C was selected for pyrolysis of the dung as biochar produced at this temperature had a greater surface area and cation exchange capacity than biochar produced at 400°C (Table 1). The biochar was hand-crushed, passed through a 1 mm mesh screen, and 12 g were dispersed in 10 mL distilled water. The three additives that were compared with dung biochar were prepared as follows: 1.5 g *Acremonium* cellulase was dissolved in 10 mL distilled water, while *Lactobacillus plantarum* and *L. buchneri* (Vita Plus

Co, Ltd, Madison, WI, USA) were cultured in deMan Rogosa Sharpe (MRS) medium (Zheng et al., 2012) and then were centrifuged and re-suspended with sterile distilled water to an equivalent of 10 mL/kg FW (adjusted to the number of live bacteria to 1 × 10^8 CFU/mL). Additives were applied to the sweet sorghum prior to ensiling as follows: (1) deionized water, without any additives (control); (2) yak dung biochar at 40 g biochar/kg dry matter (DM) sorghum; (3) *Lactobacillus buchneri* bacteria at 1×10^6 colony forming units (CFU)/g fresh weight (FW); (4) *Lactobacillus plantarum* bacteria at 1×10^6 CFU/g FW; and (5) *Acremonium* cellulase (Rujie Bio-tech Co, Ltd, China) at 5 g/kg fresh matter (FM). A randomized design was used with three replicates for each treatment. The additives were sprayed on 300 g of chopped sweet sorghum and mixed thoroughly while an equal volume of sterile distilled water was sprayed onto the control sorghum. Subsequently, the sweet sorghums were vacuum-sealed in polythene bags (dimensions 45 × 25 cm) and maintained for 90 days at a temperature of 25 ± 3°C. All silages were cut in a commercial food processor (Robot Coupe, Co Ltd, Burgundy, France) to a size of 1 to 4 mm, vacuum-sealed in 30 cm × 40 cm plastic bags and frozen at - 20°C.

2.3 In vitro incubation with rumen fluids

Rumen fluid was collected prior to morning feeding from three 2.5 year old Simmental steers (average body mass 420 kg) that were consuming 3.4 - 4.5 kg day^{-1} dry matter corn stalk. A flexible oral stomach tube (Anscitech Co. Ltd., Wuhan, China) was used to collect 100 mL of rumen fluid (Shen et al., 2012), of which the first 30 mL were discarded to minimize contamination from saliva. The fluid was filtered through four layers of cheesecloth into a pre-warmed (39°C) buffer solution under anaerobic conditions and used for gas production measurements by the Hohenheim Gas method (Menke et al., 1979). Sorghum silage samples, each of 400 mg dry matter, were incubated in triplicate in 100 mL calibrated glass fermentation tubes (Model Fortuna, Haberle Labortechnik, Lonsee-Ettlenschei β, Germany) to which 30 mL of incubation media (prepared following Menke and Steingass, 1988) were added. The glassware was maintained in a 39°C shaking water-bath for 72 h and flushed with CO_2 before use. Gas production was recorded by piston movement, after correcting for gas production due to rumen fluid alone, at 2 h, 6 h, 12 h, 24 h, 48 h and 72 h. A gas sample was collected for methane analysis from each syringe using a vacuum vessel at 12 h, 24 h, 48 h and 72 h. All gas samples were stored at -20°C.

The model of Blümmel et al. (2003) fitted cumulative gas production as:

$$Y = A\,(1 - e^{-ct}) \qquad (1)$$

Where: Y = cumulative gas volume at time t; A = asymptotic value *of* gas production; and c = rate constant of gas production. Kinetics of total gas production was estimated using the software Fig P (Biosoft, Cambridge, UK). To determine the maximum potential CH_4 yield per g of volatile solids (VS) of sorghum silage during anaerobic digestion, the biomethane potential (BMP) was estimated as (Triolo et al., 2011):

$$BMP = (VFA{*}373 + Lipid{*}1014 + Protein{*}496 + Carbohydrate{*}415 + Lignin{*}727){*}0.001 \quad (2)$$

with BMP as CH_4 NL (kg VS)$^{-1}$, and all variables as as g (kg VS)$^{-1}$.

2.4 Analytical methods

Samples of 20 g were collected from each silage treatment, diluted with 180 mL autoclaved, distilled water, and then stirred for 0.5 min in a blender. The samples were filtered through four layers of cheesecloth, and pH was measured (pH meter, Hanna Instruments, Italia Srl, Padova, Italy). Two 20 mL samples were each placed in a 50 mL polypropylene centrifuge tube; one sample for NH_3-N concentration determination (Broderick and Kang, 1980) and one was acidified with H_2SO_4 (7.14 M). Samples were filtered using a 0.22 µm dialyzer to determine water-soluble carbohydrates (Gao et al., 2008). Volatile fatty acids (VFA), including lactic, acetic, propionic and butyric acids, were determined at the end of each incubation (72 h). Briefly, rumen fluid from each syringe was collected in 10 mL centrifuge tubes, placed in liquid nitrogen and then stored in an ultra-low temperature freezer. Six mL of fluid were centrifuged at 3,000 × g for 10 min and, subsequently, 1 mL of supernatant and 0.2 mL of 25% H_3PO_4 containing 2 g L^{-1} internal standard substances (2-ethyl butyraldehyde) were added in a 1.5 mL centrifuge tube, placed in ice water for half an hour, and centrifuged at 10,000 × g for 10 min at 4°C (Zhang et al., 2016). The VFAs were analyzed using an Agilent HPLC 1260 (KC-811 column, Shodex; Shimadzu, Kyoto, Japan) with a column temperature of 50 °C, carrier gas of helium with a flow rate of 1.0 mL min^{-1} and a detection wavelength of 210 nm.

Fresh sorghum and silage samples were freeze-dried (Freeze Dryer-1A-50, Boyikang, Beijing, China) and ground to pass through a 1 mm screen. Dry matter content was determined as the difference between fresh and freeze-dried silage, dry matter loss as the difference in dry matter before and after silage, ash by combustion of a sample in a muffle furnace at 550°C for 8 h (AOAC, 2001; method 990.03), neutral/acid detergent fiber as outlined by Van Soest et al. (1991) and water-soluble carbohydrates by high performance liquid chromatography (Gao et al., 2008). Nitrogen was determined by the Kjeldahl method (AOAC, 2001) and crude protein as Kjeldahl N × 6.25. Gross energy was measured by automatic adiabatic bomb calorimetry following the manufacturer's protocol (KT-R4300, Kaite Co. Ltd., China). Methane was determined by injecting 100 uL gas sample into a SP-3420A series gas chromatograph (Beijing Beifen-Ruili Analytical Instrument (Group) Co., Ltd.), equipped with a hydrogen flame ionization detector (Zhang et al., 2016). The incubated bottle was opened, and the content was filtered through a glass filter crucible, dried in an oven at 100°C for 24 h and weighed for *in vitro* DM digestibility (IVDMD) determination.

2.5 Statistical analysis

Data were analyzed by ANOVA using the SAS package (SAS Institute Inc., Cary, NC, USA, version 6.12). Significance was accepted at $P < 0.05$ and a *post-hoc* Tukey test separated means where significance existed.

3. Results and Discussion

3.1 Silage composition

Dry matter content of sweet sorghum prior to ensiling was 234 g/kg fresh matter while the water-soluble carbohydrate concentration was 116 g/kg DM. Neutral and acid detergent fiber contents were 538 and 306 g/kg DM, respectively, crude protein was 102 g/kg DM; ash content was 105 g/kg DM and gross energy was 17.3 MJ/kg DM. Thus, sweet sorghum contained a high level of water-soluble carbohydrates content, which is essential for good quality silage (Figure 4).

The DM content of the treated silages were significantly ($P < 0.05$) lower than the control silage, except for the *L. plantarum* treatment, which had the greatest DM content. In addition, *L. plantarum* treatment underwent greater homolactic fermentation than the other silages, thereby reducing DM loss during ensiling (Liu et al., 2017). The *L. plantarum*-treated silage had the greatest crude protein content ($P < 0.05$) and the greatest concentration of lactic acid (84.8 g/kg DM), which lowered the pH (3.89). It was reported that the abundance of Clostridia decreased with *Lactobacillus*-treated silages due to the high lactic acid content produced (Tabacco et al., 2009; Cai et al., 1998). The silage with yak dung biochar had high lactic acid content while the biochar did not provide an appropriate pore size and habitat for clostridia (0.3 - 13 µm) to proliferate (Luz et al., 2018), suggesting a low clostridia abundance with the biochar additive. This would ultimately decrease crude protein loss (Nadeau et al., 2000), as clostridia produce ammonia nitrogen from decomposed protein in silage (Xing et al., 2009). The increase in DM degradation of silage with *Acremonium* cellulase could be attributed to the enzymatic hydrolyzing activity of the microbes (Borreani et al., 2018).

Silage with biochar had significantly lower neutral detergent (587 vs. 635 g/kg DM; $P < 0.001$) and acid detergent fiber (343 vs. 359 g/kg DM; $P < 0.001$) contents and a higher digestibility of these fibers by 8% and 4%, respectively, than the control silage. EELS of yak dung biochar showed high functionality, especially C=O and C-O groups (Figure 3), which contribute to small amounts of lignin, cellulose and hemicellulose (Luz et al., 2018). By comparison, *Acremonium* cellulose-treated silage had a 14% and 12% greater digestibility of neutral and acid detergent fiber, respectively, than control silage (Figure 4). The increased neutral/acid detergent fiber digestibility of the cellulase-treated silage was related to the digestion of cellulose by cellulase during ensiling, leaving the less-digestible lignin and hemicellulose for microbial degradation in the rumen (Nadeau et al., 2000). In contrast, Khota et al. (2017) reported that cellulase had no effect on fiber digestibility in sorghum (*bicolor* cv. IS 23585) silage, because of a sharp decrease in pH, which led to an inhibition of cellulase activity.

Biochar-treated silage had a greater gross energy yield than the control (17.8 vs. 17.4 MJ/kg DM; $P < 0.001$) and ranked highest among all treatments (Figure 4). The gross energy in silage is an important quality factor (DePeters et al., 2000). Furthermore, biochar-treated silage had greater quantities of ($P < 0.001$) water-soluble carbohydrates than all treatments, while the silages with commercial additives had lower water-soluble carbohydrate content than the control. This finding was consistent with Jindo et al. (2016), who reported high levels of carbohydrates extracted from compost treated with biochar. High water-soluble carbohydrate content is desirable for silage, as it supplies substrates for bacteria to produce VFAs that reduce pH and improve storability of silage (Weiland, 2010). When energy is limiting but there is an excess of carbohydrates in the rumen, more non-protein N and amino acids can be used by microbes to synthesize microbial proteins. Biochar-treated sorghum silage, with high water-soluble carbohydrates, therefore, improves the C and N balance (Miller et al., 2001), which increases rumen microbial protein production (Parsons et al., 2011). Although modes of action of biochar in silage production are still unclear, intensive studies of biochar properties are planned to reveal the potential role of biochar as a silage additive.

3.2 Digestibility, gas and methane production

In vitro DM digestibility (IVDMD) and gas and methane production of sorghum silage after 90 days of incubation are presented in Table 2. It was expected that biochar-treated silage would have a higher IVDMD than control silage. It is well established that biochar provides a surface area and mineral nutrients that promote the formation of a microbial biofilm (Figure 2), which can stimulate rumen microbial activity and improves ruminal feed digestion (Leng, 2014). However, the digestibility with biochar (6.6% of dietary DM in this study) was similar to the control suggesting that biofilm formation and activity did not play a critical role in our study. Further research is required to identify the role and contribution of biochar biofilm on IVDMD. Similarly, Hansen et al. (2012) reported that IVDMD was not affected when straw biochar was included at 9% dietary dry matter. However, biochar from bamboo at 5% dietary DM improved apparent DM digestibility in goats fed a grass and concentrate mixture (Van et al., 2006). A high level of biochar may disturb rumen metabolism by increasing the amount of inactive material in the diet (Van et al., 2006) and, therefore, a lower level of biochar may be preferable in some cases.

The total gas production of the biochar treated-silage and control silage was 1.3-4.0 times greater ($P < 0.001$) than in the other three treatments (Table 2), which would indicate that the metabolizable yield was also higher (Menke and Steingass, 1988). Cumulative gas production profiles from all silages are presented in Figure 5 and the predicted parameters are presented in Table 3. After 72 h, gas production varied from 30.0 to 120 mL per 400 g of silage DM. Gas production and the estimated potential total gas yield of *L. buchneri* treated silage were 4 times lower ($P < 0.001$) than in the other silages at all incubation periods.

The difference in methane emission among treatments became evident after 12 h incubation and the cumulative production of *L. buchneri*-treated silage was the lowest (Figure 5). The BMP test, however, indicated the potential CH_4 yield from *L. buchneri*-treated silage was higher than in the controls (Table 3). It was reported that the calculated BMP can differ substantially from the true measurements as occurred in the present study. The *in-vitro* degradation of *L. buchneri*-treated silage may have been limited by biodegradability and ultimate production of inhibitors (Teghammar, 2013).

Methane production and pH at 72 h did not differ between biochar-treated and control silage (Figure 5; Table 2), which was supported by a previous study in which biochar did not affect gas production (Pereira et al., 2014). However, it was expected that methane would be reduced in biochar-treated silage, as it was reported that biochar can reduce ruminal enteric methane emissions by decreasing rumen methanogens and increasing methanotrophs (Toth et al., 2016). Furthermore, the ability of biochar to decrease methane emission was linked to an increase in methanotrophs relative to methanogens in rice paddy soils where methane emission was reduced (Feng et al., 2012). However, Mumme (2014) reported that alkaline biochar enhanced methane production by increasing pH as a result of the conversion of CO_2 to HCO_3^- or CO_3^{2-}. The stability can be improved by increasing the buffering capacity through pH reduction by VFAs. Differences in digestibility and methane production among studies in which biochar was added may be due to the source of the biomass for the biochar, particle size, and pyrolysis temperature and conditions, as they can alter rumen fermentation (McFarlane et al., 2017). When biochar is produced using lower temperatures for pyrolysis, the specific surface area is reduced and, consequently, its ability of nutrient uptake and to supply a habitat for the formation of biofilm is reduced (Leng, 2014). However, biochar produced at lower temperatures has a greater volatile matter content, which serves as a carbon and energy source and, thus, promotes microbial growth (Crombie et al., 2013).

3.3 Silage fermentation products

The quality of the sorghum silages is shown in Figure 6. All silages had acidic pH values

(3.89 - 4.24). The high content of water-soluble carbohydrates (116 g/kg DM) allowed the lactic acid bacteria to produce high concentrations of lactic acid (Khota et al., 2017). This acid was likely the main reason for the drop in pH due to its strong acidity (pKa of 3.86) (Herrmann et al., 2011). In this study, although biochar-treated silage had a higher concentration of lactic acid than the control (Figure. 6), it also had a higher pH ($P < 0.05$), most likely as a result of the high ash content of the dung and high pH (10.6) of the biochar (Table 1). The high pH is not necessarily indicative of poor fermentation of silage, but silage from restricted fermentation can be unstable when exposed to air. Butyric acid content was below detection (< 0.01 g/kg DM), which is beneficial, because if butyric acid concentration exceeds 5 g/kg of DM in silage, it can contribute to clostridial fermentation. However, the presence of moderate amounts of butyric acid improves aerobic stability of untreated forages (Adesogan et al., 2004). The high concentrations of lactic acid and the absence of butyric acid in all silages suggested that no undesirable secondary clostridial fermentation occurred.

Biochar-treated silage exhibited higher concentrations of NH_3-N (20.5 vs. 13.0 g/kg TN, $P = 0.002$), lactic acid/acetic acid ratio (1.70 vs. 0.73, $P < 0.001$), and propionic acid (48.0 vs. 43.6 g/kg DM, $P < 0.001$) than the control silage. The higher NH_3-N concentration was likely due to the higher N content of manure-based biochars (Rombola et al., 2015). High contents of ammonia are attributed to enhanced protein degradation, which can result from a reduction of pH. Low NH_3-N concentration (< 25 g/kg DM) was reported in sorghum straw silage treated by enzymes and inoculant plus enzymes (Xing et al., 2009). The enzyme treatment contributed to a sharp decline in pH, which inhibited aerobic microbes and plant enzymes, resulting in a decrease in protein breakdown in the incubation process.

Acetic acid is an important fermentation end-product with a typical concentration of approximately 40 g/kg DM (Kleinschmit and Kung, 2006). A high concentration of acetic acid generally results in weak dry matter and energy recovery, but low acetic acid concentration cannot maintain aerobic stability (Xing et al., 2009). In the present study, acetic acid content in all treatments ranged from 24.0 to 50.8 g/kg DM and was, therefore, suitable for maintaining aerobic stability. The content of acetic acid was lowest in the biochar-treated silage ($P < 0.001$), which indicated that a less heterolactic process of epiphytic microbes occurred in this silage (Li et al., 2019). *Lactobacillus buchneri*, *Acremonium* cellulase and control treatments resulted in lower lactic to acetic acid ratios than the biochar treatment (0.93, 1.18 and 0.73 vs. 1.70, respectively; $P < 0.01$) (Figure 6), indicating that biochar-treated silage underwent more homo-fermentation.

A cost comparison was done to determine the financial benefits of using biochar compared with commercial silage additives (Table 4). Using the current average costs at production, biochar would cost US $9.78 for a ton of sorghum forage compared with US $94 to $125 per ton for commercial additives (Shackley and Clare, 2015). This is a substantial saving for herders in Tibet and remote regions, which could make this option feasible for them to use. The low price would make biochar attractive as an ensiling agent on the world market.

4. Conclusions

Yak dung biochar added to ensiled sweet sorghum increased concentrations of crude protein, lactic acid, and water-soluble carbohydrates and also increased gross energy yield. Therefore, the silage quality was improved with the addition of yak dung biochar, which supported the initial hypothesis. Cost benefit analysis showed that the biochar application in silage production was approximately one tenth the costs of commercial inoculants; consequently, yak dung biochar is a novel low-cost additive that would be affordable by Tibetan herders. Therefore, the second hypothesis was supported as well. More prebiotic (lactic acid) was produced in ensilaged food in the presence of biochar as a biosecurity measure. Biochar-treated silage can have a large impact on farmers using sustainable farming practices in remote regions. The potential profit from this new enterprise could offset the loss of dung as fuel and improve the health of the Tibetan people by decreasing the hazardous use of dung for heating and cooking in the home.

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Figure 1. (a) Collecting and stacking of yak dung near a Tibetan home (Photograph by A. Allan Degen). (b) Inside the home of a Tibetan herder using yak dung for heating and cooking (Photograph by Yanfu Bai). (c) Production of biochar.

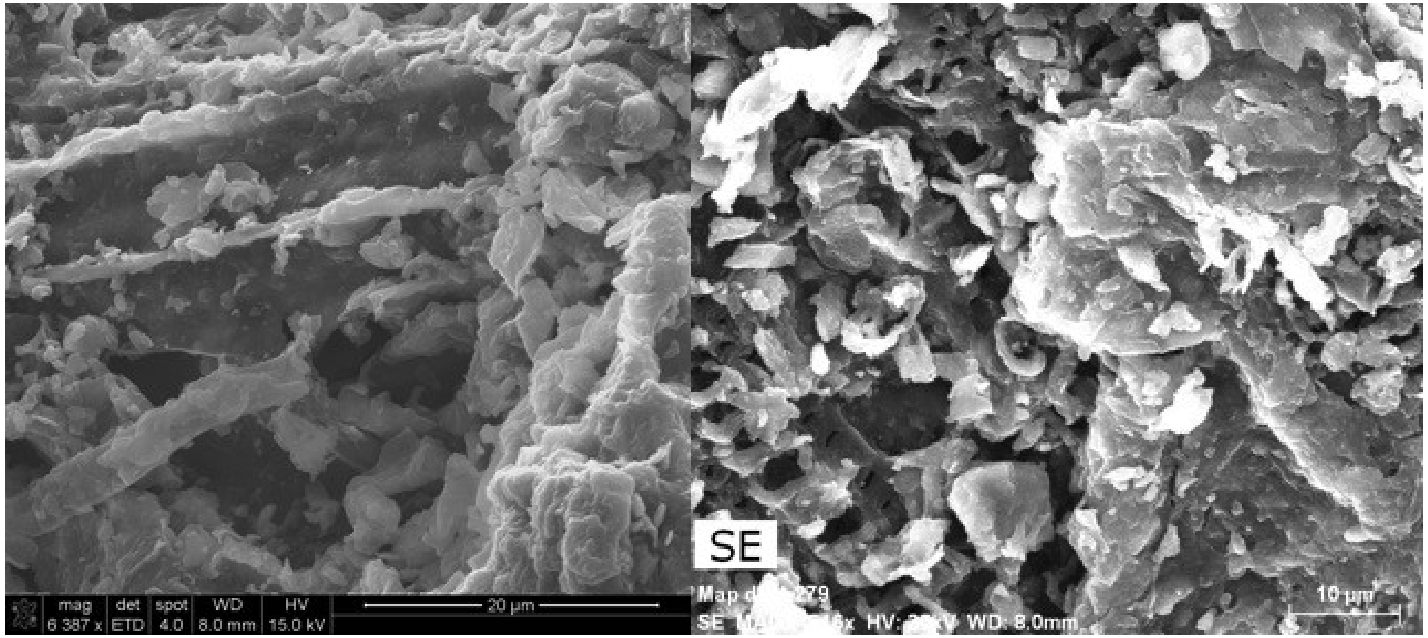

Figure 2. Scanning electron microscope (SEM) image of yak dung biochar pyrolysed at 500°C.

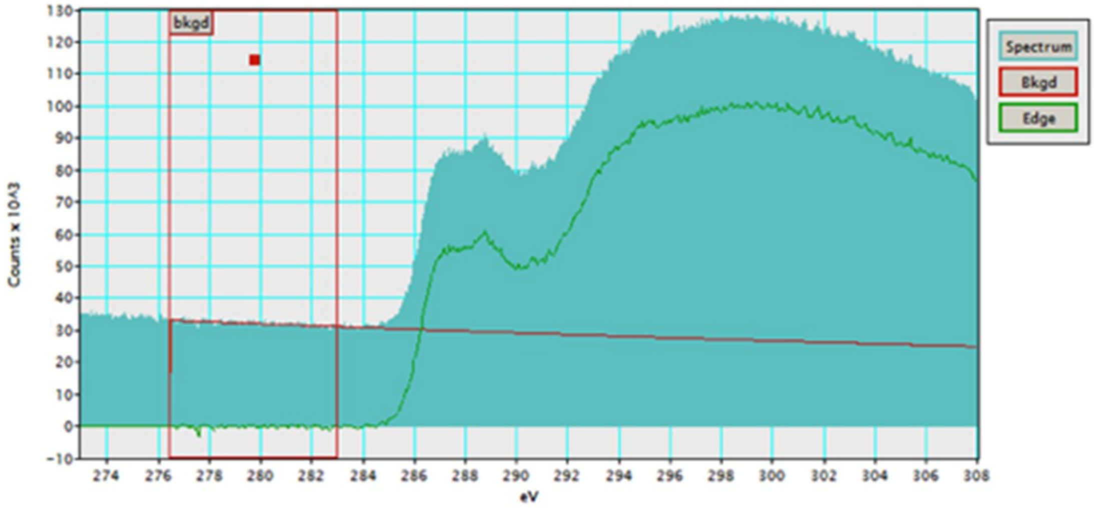

Figure 3. Carbon electron energy loss spectrometry of yak dung biochar pyrolysed at 500°C with a holding time of 2 hours.

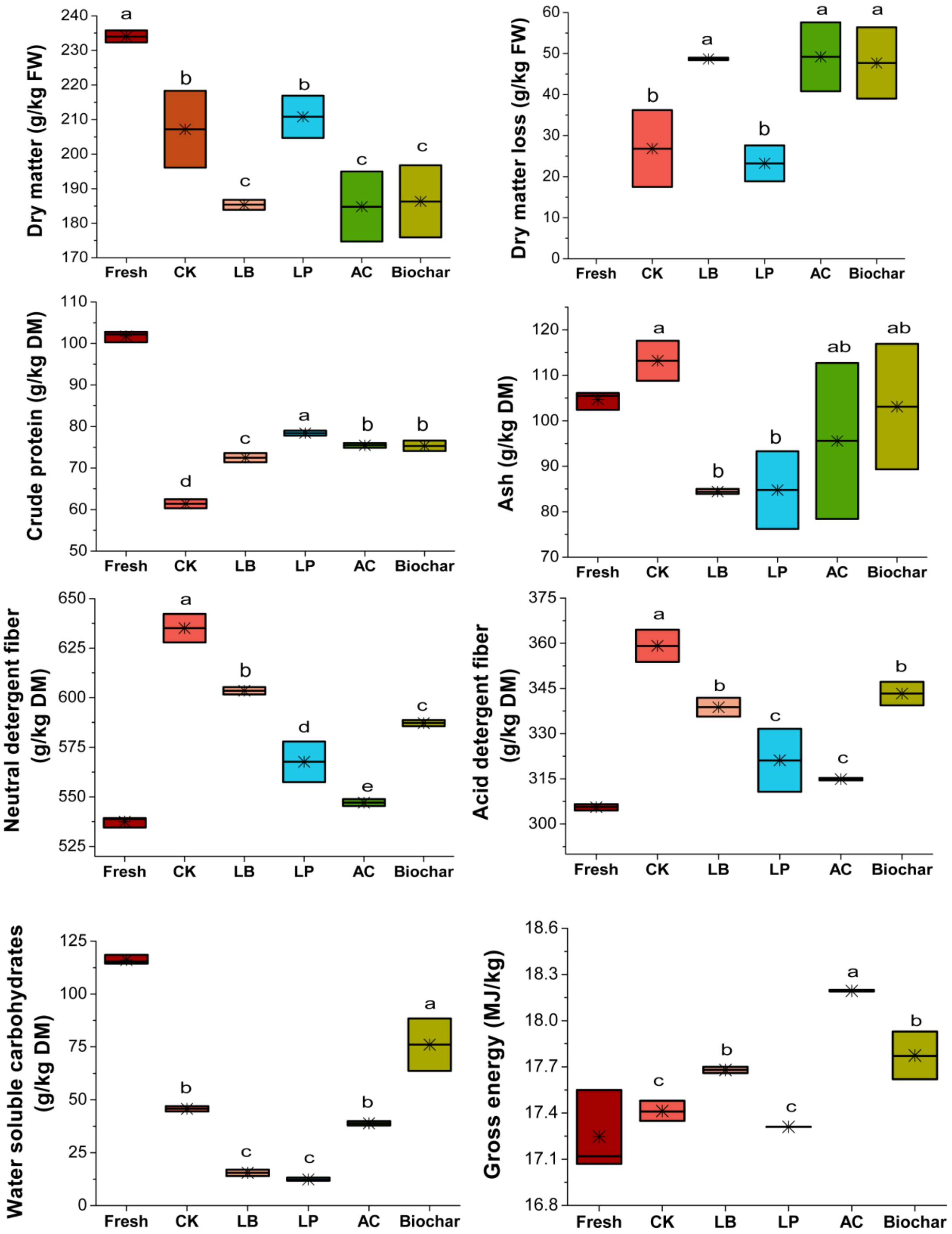

Figure 4. Chemical composition, water-soluble carbohydrates and gross energy of sorghum silages after 90 days of fermentation. CK, control; LB, *Lactobacillus buchneri*; LP, *Lactobacillus plantarum*; AC, *Acremonium cellulose*; Biochar, produced from yak dung; Means with different letters differ significantly from each other ($P < 0.05$).

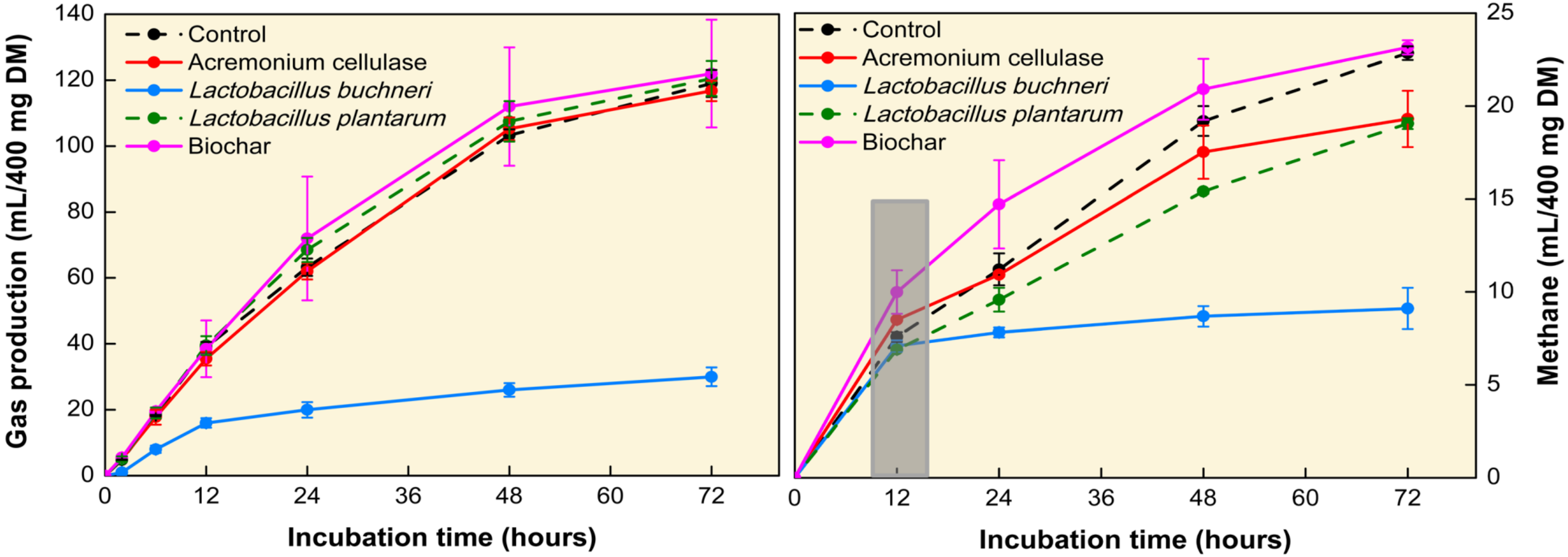

Figure 5. Effect of additives on *in vitro* total gas production and methane emission of sweet sorghum silage.

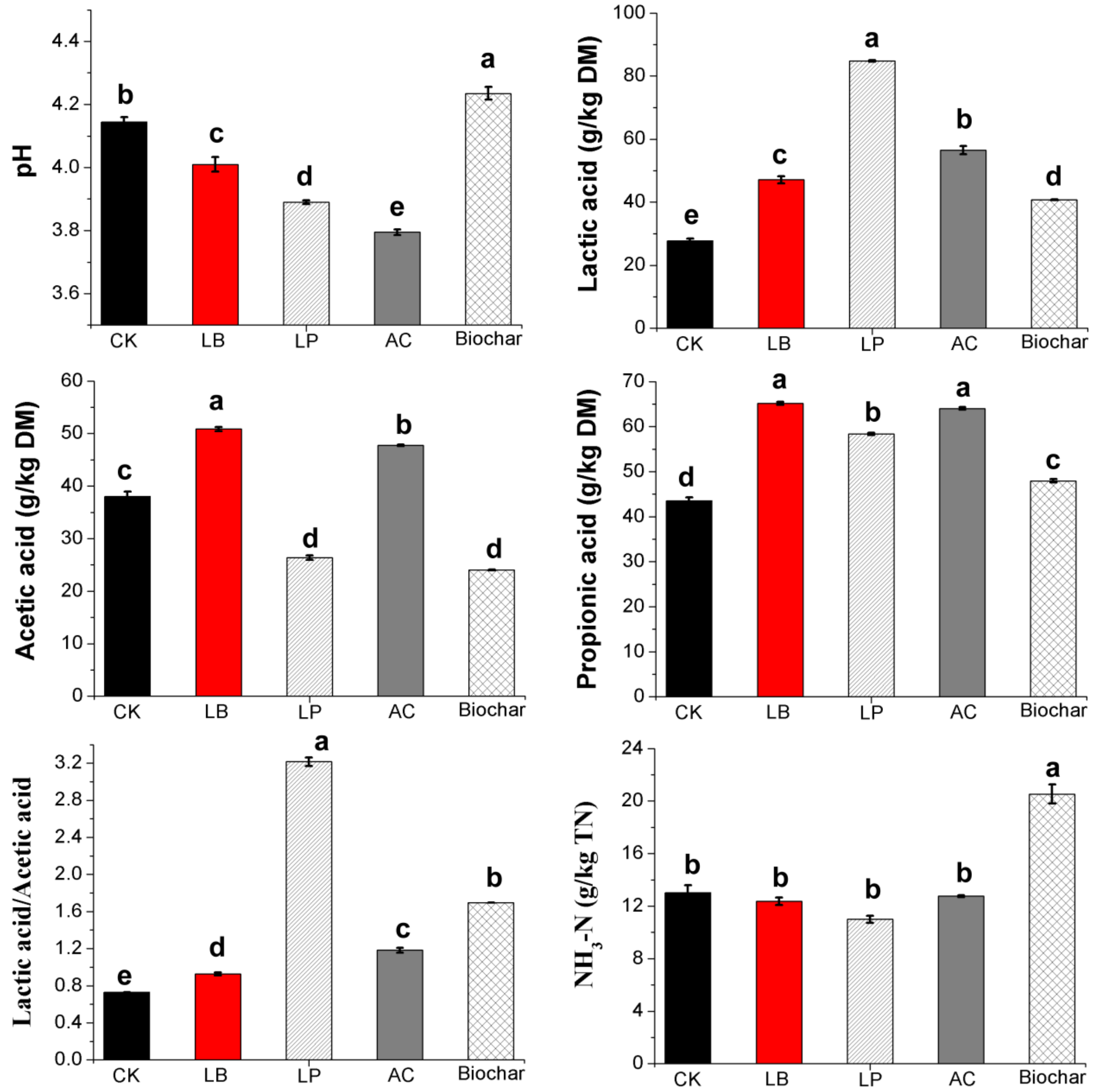

Figure 6. Volatile organic acid concentrations of sorghum silages after 90 days of fermentation. DM, dry matter; Butyric acid not detected; CK, control; LB, *Lactobacillus buchneri*; LP, *Lactobacillus plantarum*; AC, *Acremonium cellulose*; Biochar, produced from yak dung. Means with different letters differ significantly from each other ($P < 0.05$).

Table 1 Main characteristics of starting materials (yak dung) and biochar type obtained by slow pyrolysis at 400°C and 500°C.

Properties	Yak dung	Biochar	
		Yak dung (400°C)	Yak dung (500°C)
pH (/)	7.34	10.1	10.6
Surface area (m^2/g)	ND	3.02	6.99
Average pore size (nm)	ND	14.5	8.50
Cation exchange capacity (Meq /100 g)	ND	45.2	66.5
Anodic capacitance (F/g)	ND	7.5	18.4
Cathodic capacitance (F/g)	ND	25.6	13.7
Composition (% dry matter)			
Ash	25.8	40.9	45.2
Carbon	30.3	43.6	46.9
Nitrogen	1.53	1.76	1.72
Hydrogen	4.88	3.07	1.84
Oxygen	37.5	10.7	4.34
Iron	1.06	1.07	1.09
Potassium	1.07	1.42	1.82
Phosphorous	0.19	0.29	0.38
Manganese	0.04	0.04	0.04

Note: ND, not determined; DM, dry matter. (Rafiq et al., 2017).

Table 2 IVDMD, gas production, and methane emission at 72 hours of sorghum silages after 90 days of fermentation.

Items	IVDMD (g/kg)	pH (/)	GP (L/kg DM)	Methane production		
				(mL/L GP)	(L/kg DM)	(L/kg IVDMD)
CK	577^{b}	6.91^{a}	511^{a}	194^{b}	57.1^{a}	171^{a}
LB	581^{b}	6.72^{c}	127^{c}	310^{a}	22.8^{c}	67.7^{b}
LP	751^{a}	6.84^{b}	400^{b}	158^{c}	47.7^{b}	84.3^{b}
AC	776^{a}	6.85^{ab}	376^{b}	129^{d}	48.3^{b}	62.5^{b}
BC	605^{b}	6.88^{ab}	507^{a}	205^{b}	57.9^{a}	171^{a}
SE	23.6	0.018	38.7	16.8	3.44	13.6
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note: IVDMD, in vitro dry matter digestibility; GP, gas production; DM, dry matter content; GE, gross energy; CK, Control; LB, *Lactobacillus buchneri*; LP, *Lactobacillus plantarum*; AC, *Acremonium cellulose*; BC, biochar produced from yak dung; SE, standard error of the mean (n = 3). Means in the same column with different uppercase letters differ significantly from each other ($P < 0.05$).

Table 3 Kinetics of in vitro total gas production after 72 h incubation of the sweet sorghum silage and biomethane potential (BMP) as affected by different additives.

Items	A (mL/400 mg DM)	c (mL/h)	BMP (CH_4 NL (kg VS)$^{-1}$)[1]
CK	145 ^{a}	0.03 ^{b}	154 ^{d}
LB	29.1 ^{b}	0.06 ^{a}	167 ^{c}
LP	142 ^{a}	0.03 ^{b}	171 ^{bc}
AC	146 ^{a}	0.03 ^{b}	180 ^{a}
BC	150 ^{a}	0.03 ^{b}	175 ^{b}
SE	13.2	0.004	2.41
P-Value	< 0.001	< 0.001	< 0.001

[1] Lipid and lignin content in calculation taken from unpublished data. Note: CK, control; LB, *Lactobacillus buchneri*; LP, *Lactobacillus plantarum*; AC, *Acremonium cellulose*; BC, biochar produced from yak dung; SE, standard error of the means; BMP, biomethane potential; NL, norm liter (273 K, 1.013 bar); VS, volatile solids. Means in a column with different superscripts differ significantly from each other ($P < 0.05$).

Table 4 Cost evaluation of biochar additive compared with commercial silage agents.

Additives	Source	Additive dose (kg/ton sorghum forage)	Price (US $/kg)	Cost (US $/ton)
Lactobacillus buchneri	Vita Plus corporation, USA	5.00	25.0	125
Lactobacillus plantarum	Vita Plus corporation, USA	5.00	20.0	100
Cellulase	Rujie Bio-tech corporation, China	5.00	18.8	94.0
Biochar	Pyrolyzed from Tibetan Yak dung	12.0	0.815 (average)	9.78

Note: Biochar additive applied at 4% DM. Commercial silage additives are dosed at 0.5 % fresh weight basis. To estimate the price of commercial biochar, a survey was carried out. Chinese bamboo biochar producer SEEK is selling it at between 400-800 US $/ton; the factory gate purchase price of biochar from domestic sources in Europe is 600-1200 US $/ton; Sonnenerde in Austria, selling biochar to farmers at a price of 600 US $/ton; Biochar in Switzerland is sold at 905 US $/ton; Yorkshire Charcoal in the UK is sold at 1200 US $/ton (Shackley and Clare, 2015). The average price of biochar was 815 ± 308 US $/ton. *Lactobacillus buchneri, Lactobacillus plantarum,* and cellulase were imported by the Sanger Biotechnology Corporation, Ltd, Shanghai city, China in 2016.