Postprint: In Vitro Rumen Fermentation Characteristics of Mixed Potato Vine Silage Using Rumen Simulation Technology

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Abstract

This study investigated the in vitro rumen fermentation characteristics of diets containing potato stem and leaf mixed silage using the rumen simulation technique (Rusitec). A single-factor completely randomized design was employed, dividing the 8 fermentation vessels of the Rusitec system into 2 groups. Corn straw silage and potato stem and leaf mixed silage (ensiled mixture of potato stems and leaves with corn straw at a 40:60 ratio) were used to formulate fermentation substrates for sheep diets in the two groups, respectively. Each group comprised 4 replicates, with each replicate consisting of 1 fermentation vessel. The experiment included an 8-day adaptation period and a 2-day sampling period. The results demonstrated that the potato stem and leaf mixed silage group had significantly higher gas production, total nitrogen, ammonia nitrogen (except at 3 h post-feeding) and microbial nitrogen contents in the fermentation fluid throughout the entire period, and propionate content in the fermentation fluid at 6 and 9 h post-feeding compared to the corn straw silage group ($P<0.05$). Conversely, methane production, pH of fermentation fluid at 3 h post-feeding, acetate content in fermentation fluid at 9 h post-feeding, and acetate/propionate ratio in fermentation fluid at 6 and 9 h post-feeding were significantly lower in the potato stem and leaf mixed silage group ($P<0.05$). No significant differences were observed in nutrient degradation rates or other rumen fermentation parameters between the two groups ($P>0.05$). It can be concluded that potato stem and leaf mixed silage exerted no adverse effects on in vitro rumen fermentation in sheep, and was beneficial for reducing environmental pollution from rumen methane while promoting energy and nitrogen utilization in ruminants.

Full Text

A Study on in Vitro Rumen Fermentation Characteristics of Potato Vines and Leaves Mixed Silage Based on Rumen Simulation Technique

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Abstract

This study investigated the in vitro rumen fermentation characteristics of potato vines and leaves mixed silage using rumen simulation technique (Rusitec). A complete one-factor randomized design was employed, dividing eight fermentation tanks of the Rusitec system into two groups (four replicates per group, one tank per replicate). Corn stalk silage and potato vines and leaves mixed silage (prepared by mixing potato vines and leaves with corn stalk at a 40:60 ratio) were used to formulate sheep diets, which served as fermentation substrates for their respective groups. The experiment comprised an 8-day pre-test period and a 2-day sampling period. The results demonstrated that compared with the corn stalk silage group, the potato vines and leaves mixed silage group exhibited significantly higher gas production, total nitrogen content in fermentation fluid throughout the entire period, ammonia nitrogen content (except at 3 h post-substrate replacement), microbial nitrogen content, and propionic acid content in fermentation fluid at 6 and 9 h post-substrate replacement ($P<0.05$). Conversely, methane production, fermentation fluid pH at 3 h post-substrate replacement, acetic acid content in fermentation fluid at 9 h post-substrate replacement, and acetic acid/propionic acid ratio in fermentation fluid at 6 and 9 h post-substrate replacement were significantly lower ($P<0.05$). No significant differences were observed between the two groups in nutrient degradation rates or other rumen fermentation parameters ($P>0.05$). These findings indicate that potato vines and leaves mixed silage does not adversely affect in vitro rumen fermentation in sheep, while simultaneously reducing ruminal methane pollution and promoting energy and nitrogen utilization in ruminants.

Keywords: potato vines and leaves; silage; rumen fermentation; rumen simulation technique

Potato vines and leaves represent a high-yielding agricultural byproduct in China and worldwide. With relatively high crude protein (CP) content [1-2], calcium (Ca) and phosphorus (P) levels [2-3], and low crude fiber content [1-3], they constitute a promising feed resource. Potato vines and leaves can be processed into silage [1-9], which improves palatability without causing harm to ruminants [10-11]. However, research on the effects of potato vines and leaves silage on ruminant rumen fermentation remains limited. Only Malecky et al. [12] have

investigated the impact of potato vines and leaves silage supplemented with 5% molasses and 1.5% urea on sheep rumen fermentation using in vitro gas production methods, reporting no adverse effects on rumen fermentation. To further elucidate the effects of potato vines and leaves silage on ruminants, this study utilized rumen simulation technique (Rusitec) to examine the in vitro rumen fermentation characteristics of potato vines and leaves mixed silage, aiming to provide a theoretical foundation for the research and application of this feed resource.

1.1 Experimental Design

A complete one-factor randomized design was implemented, allocating the eight fermentation tanks of the Rusitec system into two groups. Corn stalk silage and potato vines and leaves mixed silage (prepared by mixing potato vines and leaves with corn stalk at a 40:60 ratio) were used to formulate sheep diets, which served as fermentation substrates for their respective groups, with four replicates per group and one fermentation tank per replicate.

1.2 Fermentation Substrate Formulation

The concentrate-to-forage ratio of the fermentation substrate was 40:60. Corn, soybean meal, alfalfa hay, and wheat straw were sourced from the dairy farm of Gansu Agricultural University. Wheat straw was chopped to 2–3 cm lengths, and all ingredients were in feedable condition. Both types of silage were prepared in our laboratory. The quality parameters of the potato vines and leaves mixed silage were: pH 4.87; ammonia nitrogen (NH3-N) content 21.98 g/kg TN (total nitrogen); dry matter (DM) content 168.82 g/kg; lactic acid (LA) content 23.89 g/kg; CP content 114.28 g/kg; neutral detergent fiber (NDF) content 556.13 g/kg (LA, CP, and NDF contents on DM basis). The quality parameters of corn stalk silage were: pH 4.38; NH3-N content 18.77 g/kg TN; DM content 197.98 g/kg; LA content 31.61 g/kg; CP content 62.75 g/kg; NDF content 677.30 g/kg (LA, CP, and NDF contents on DM basis). The composition and nutrient levels of the fermentation substrates are presented in Table 1 .

1.3 Rusitec System Conditions

The Rusitec system was manufactured by Shina-gawa (Japan). Buffer solution was prepared according to McDougall [13] with the following composition: NaHCO3 9.8 g, Na2HPO4·12H2O 9.3 g, NaCl 0.47 g, KCl 0.57 g, MgCl2 0.06 g, and CaCl2 0.04 g, diluted to 1,000 mL with distilled water. Rumen fluid was collected from three 9-month-old Small-tailed Han sheep [(body weight 20.88±1.75) kg] fitted with permanent rumen fistulas. Each nylon bag (8 cm×17 cm, pore size 100 m, prepared according to reference [14]) contained 12 g of fermentation substrate (DM basis). Buffer flow rate was 0.39 mL/min [15-17], the screen mesh size at the outflow port was 1.2 mm, mixer stirring frequency was 4–5 cycles/min, fermentation tank temperature was maintained at 39 °C, and an anaerobic environment was preserved by CO2 infusion.

1.4 Fermentation Substrate Incubation

The experiment included an 8-day pre-test period and a 2-day sampling period. On day 1 of the pre-test period, before morning feeding, rumen fluid and digesta from different rumen locations were collected. The rumen fluid was filtered through four layers of cheesecloth, placed in a preheated thermos flask, infused with CO2, and immediately transported to the laboratory. Rumen fluid and buffer solution were distributed into eight preheated fermentation tanks installed in a 39 °C water bath (400 mL rumen fluid + 400 mL buffer solution). At 09:00, one nylon bag containing 70 g of digesta and one nylon bag containing 12 g of fermentation substrate were placed in each fermentation tank, and CO2 was infused into the tanks. At 09:00 on day 2 of the pre-test period, the nylon bags containing digesta were removed and replaced with new nylon bags containing 12 g of fermentation substrate. At 09:00 on day 3 of the pre-test period, the nylon bags containing substrate fermented for 48 h were removed and replaced with new bags. During the sampling period, one nylon bag digested for 48 h was removed and a new nylon bag containing 12 g of fermentation substrate was inserted daily at 09:00. The substrate replacement process was completed within 30 min.

1.5 Sample Collection

Total gas production over 24 h was collected on day 1 of the sampling period for determination of gas production and methane yield. On day 2 of the sampling period, fermentation fluid was collected at 0, 3, 6, 9, and 12 h post-substrate replacement. pH was measured immediately, and samples were stored at −20 °C for subsequent analysis of total nitrogen, NH3-N, urea nitrogen, and volatile fatty acid (VFA) contents. Fermentation residues digested for 48 h were collected on day 2 of the sampling period for determination of DM, CP, NDF, and ADF contents to calculate 48 h rumen degradation rates. The degradation rate of a specific nutrient was calculated using the following formula:

Degradation rate of nutrient (%) = 100 × (content of the nutrient before fermentation − content of the nutrient after fermentation) / content of the nutrient before fermentation.

1.6 Analytical Methods

Fermentation fluid pH was measured using a pH meter (PHS-3C, Shanghai Leici Instrument Factory). Gas production was measured using a gas flow meter (DC-1, Shina-gawa, Japan). Methane production was determined by gas chromatography (6890N, Agilent, USA) using an AT.SE-30 capillary column (Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences). Fermentation fluid urea nitrogen content was measured using the diacetyl-monoxime method with assay kits purchased from Nanjing Jiancheng Bioengineering Institute. Fermentation fluid VFA content was determined by gas chromatography using a 30 m×0.32 μm×0.5 μm capillary column (Agilent, USA). Fermentation fluid NH3-

N content was measured according to the colorimetric method of Feng Zongci et al. [18]. Microbial nitrogen content in fermentation fluid was calculated as follows:

Microbial nitrogen content (mg/dL) = Total nitrogen content − NH3-N content − Urea nitrogen content [19].

Total nitrogen content in rumen fluid and DM, CP, crude ash (Ash), ether extract (EE), Ca, and P contents in fermentation substrates and residues were determined according to methods described by Yang Sheng [20]. NDF and acid detergent fiber (ADF) contents in fermentation substrates and residues were determined according to the method of Van Soest [21].

1.7 Statistical Analysis

Data were analyzed using SPSS 19.0 software with t-tests. Differences were considered significant at $P<0.05$.

2.1 Gas Production

As shown in Table 2 , gas production in the potato vines and leaves mixed silage group was significantly higher than that in the corn stalk silage group ($P<0.05$), while methane production was significantly lower ($P<0.05$).

2.2 Rumen Fermentation Characteristics

Table 3 reveals that the potato vines and leaves mixed silage group exhibited significantly lower pH at 3 h post-substrate replacement, acetic acid content in fermentation fluid at 9 h post-substrate replacement, and acetic acid/propionic acid ratio in fermentation fluid at 6 and 9 h post-substrate replacement compared with the corn stalk silage group ($P<0.05$). The potato vines and leaves mixed silage group showed significantly higher pH at 9 h post-substrate replacement and propionic acid content in fermentation fluid at 6 and 9 h post-substrate replacement ($P<0.05$). Additionally, total nitrogen, NH3-N (except at 3 h), and microbial nitrogen contents in fermentation fluid throughout the entire period were significantly higher in the potato vines and leaves mixed silage group ($P<0.05$).

2.3 Nutrient Degradation Rates

Table 4 indicates that no significant differences were observed between the two groups in DM, CP, NDF, or ADF degradation rates ($P>0.05$).

3 Discussion

Gas production serves as an indicator of feed fermentability; higher fermentability and rumen microbial activity result in greater gas production [22]. In this study, the significantly higher gas production in the potato vines and leaves

mixed silage group suggests that this substrate is more conducive to rumen fermentation. Malecky et al. [12] used in vitro gas production to compare the effects of replacing 50% alfalfa hay with potato vines and leaves silage (supplemented with 5% molasses and 1.5% urea) on rumen fermentation gas production in sheep, finding no significant effect and concluding that potato vines and leaves silage could replace 50% of alfalfa hay. During ruminal fermentation of dietary carbohydrates by bacteria and protozoa, substantial amounts of formic acid, H2, and CO2 are produced [23]. Rumen methanogens can utilize H2 and CO2 to synthesize methane, which is released into the atmosphere via eructation. This process not only causes feed energy loss but also exacerbates global greenhouse effects. The significantly lower methane production in the potato vines and leaves mixed silage group indicates that this substrate favors feed energy utilization, reducing both energy loss and atmospheric methane pollution. This result aligns with the findings of higher propionic acid content and lower acetic acid/propionic acid ratio at certain time points in this group compared with the corn stalk silage group. No comparable studies are available for further analysis.

Rumen fluid pH critically affects cellulolytic bacterial activity; the optimal pH for cellulose degradation by these bacteria is generally above 6.3. When rumen fluid pH falls below 6.3, cellulolytic bacterial activity is partially inhibited, and when pH drops below 6.0, activity is completely suppressed [24]. In this study, fermentation fluid pH values for both groups ranged from 7.02 to 7.28 at all time points, which falls within the pH range required for cellulolytic bacterial activity. This indicates that potato vines and leaves mixed silage does not negatively affect cellulolytic bacterial activity.

Higher acetic acid production during rumen fermentation is associated with increased methane production, whereas elevated propionic acid content reduces methane yield [25]. In this study, the potato vines and leaves mixed silage group exhibited significantly lower acetic acid content at 9 h post-substrate replacement and significantly higher propionic acid content at 6 and 9 h post-substrate replacement, consistent with its lower methane production. Rumen microorganisms produce proteolytic enzymes that degrade dietary CP into peptides, amino acids, and ammonia to varying degrees, which can then be used as substrates for microbial protein synthesis. The significantly higher total nitrogen content in fermentation fluid of the potato vines and leaves mixed silage group likely resulted from the higher dietary CP content in this group' s diet (146.87 g/kg) compared with the corn stalk silage group (133.27 g/kg). Rumen fluid NH3-N content depends on dietary protein content, degradation characteristics, and outflow rate. When dietary protein degradation rates are similar, protein content becomes the primary factor influencing rumen fluid NH3-N concentration [25]. The higher NH3-N content in the potato vines and leaves mixed silage group likely stemmed from higher total nitrogen content in fermentation fluid and similar CP degradation rates between groups. Satter et al. [26] reported that normal rumen fluid NH3-N concentrations range from 0.8 to 56.1 mg/dL. In this study, NH3-N concentrations at different time points (12.98–18.47 mg/dL) fell within this normal range. Rumen fluid urease activity is reportedly high,

with urea degradation to ammonia occurring four times faster than ammonia assimilation, resulting in low urea nitrogen content [27]. The low urea nitrogen contents observed in both groups, with no significant differences between them, suggest similar urease activities in fermentation fluid. Furthermore, the significantly higher microbial nitrogen content throughout the entire period in the potato vines and leaves mixed silage group resulted from higher NH3-N content in this group' s fermentation fluid.

The absence of significant differences in nutrient degradation rates between the two groups suggests that the rumen environment changes induced by the two silage types were insufficient to affect nutrient degradation. This is further supported by the lack of significant differences in total volatile fatty acid (TVFA) content between groups. Malecky et al. [12] obtained similar results using in vitro gas production, finding no significant differences in DM, NDF, or CP degradation rates between potato vines and leaves silage and alfalfa hay groups.

4 Conclusion

Potato vines and leaves mixed silage does not adversely affect in vitro rumen fermentation in sheep and offers benefits in reducing ruminal methane pollution while promoting energy and nitrogen utilization in ruminants.

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