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Effects of mixed modes on fermentation quality and *In vitro* gas dynamics of sorghum-sudangrass hybrid (*Sorghum bicolor* × *S. sudanense*) silage

Efeitos de modos mistos para a qualidade de fermentação de silagem de sorgo-sudangrass (*Sorghum bicolor* × *S. sudanense*) e características de produção de gás *in vitro*

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Abstract

Sorghum-sudangrass hybrid silage has poor fermentation characteristics owing to a high moisture content. Accordingly, a 3 × 4+1 factorial design was applied to investigate the effects of adding different types and amounts of hay (corn stalk, wheat straw, and alfalfa hay at 12.5 kg t^{-1}, 25 kg t^{-1}, 37.5 kg t^{-1}, and 50 kg t^{-1}) on the nutritive value, fermentation quality, 72 h dry matter digestibility, and gas dynamics *in vitro* to simulate the rumen fermentation of sorghum-sudangrass hybrid silage. Separated silage of sorghum-sudangrass hybrids had a high butyric acid content and a FLIEG's scores evaluation ranking of only "Fair." The addition of hay significantly improved the fermentation quality of mixed silage. With respect to hay type, adding wheat straw had the best fermentation quality, alfalfa hay had the best nutritive value, *in vitro* dry matter digestibility (IVDMD) (662.41 g kg^{-1}), *constant fractional rate* (C) *(0.28 mL h^{-1})*, and *the average gas production rate* (AGPR) (32.70 *mL h^{-1}*) content. There were no differences in *the cumulative gas production* at 72 h (GP_{72h}), *asymptotic gas production generated at a constant fractional rate* (A), and *lag time before gas production commenced* (lag) among the three hay types. With respect to quantity, 25 kg t^{-1} hay had the best fermentation quality, 50 kg t^{-1} hay had the best nutritive value and highest IVDMD content (662.81 g kg^{-1}), 37.5 kg t^{-1} hay had the highest C *(0.28 mL h^{-1})* and AGPR (31.48 *mL h^{-1}*) contents, 25 kg t^{-1} hay had the highest Half time *(2.20 h)*, and there were no significant differences in GP_{72h}, A, and lag among the four amounts. Considering both nutritive value and fermentation quality, the best mixed silage mode was 37.5 kg t^{-1} wheat straw.

Key words: Additive. By-product. *In vitro* rumen fermentation. Silage quality.

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Resumo

A fim de resolver o problema da má qualidade da fermentação de silagem causada pelo alto teor de água de sorgo-sudangrass, no exame investigou a adição de diferentes tipos de feno (talos de milho, talos de trigo e feno de alfafa) e de feno (12,5 kg t^{-1}, 25 kg t^{-1}, 37,5 kg t^{-1} e 50 kg t^{-1}) tem qual efeito para valor nutricional do armazenamento misto silagem de sorgo-sudangrass e a qualidade de fermentação de silagem e de gases in vitro. O resultado mostra, a silagem separada de híbridos de sorgo-sudangrass tinha um conteúdo de ácido butírico elevado e um ranking de avaliação de pontuações do FLIEG apenas de "Fair". A adição de armazenamento misto de feno pode melhorar significativamente a qualidade da fermentação da ensilagem de silagem de sorgo-sudangrass, do ponto de vista dos tipos de feno adicionado, o grupo de palha de trigo apresentou a maior qualidade de fermentação da silagem, e o feno de alfafa teve o maior valor nutricional, 72 h de taxa de desaparecimento da matéria seca (IVDMD) do grupo feno de índica, a taxa de produção de gás (c) e a taxa de produção de gás (AGPR) chega à taxa máxima de produção de gás foram as mais altas. Não houve diferença significativa no atraso de produção de gás (lag), produção máxima teórica de gás (A), produção cumulativa de gás de três feno às 72 h (GP_{72h}); No ponto de vista de quantidade adicionada de feno, a qualidade de fermentação da silagem do grupo de 25 kg t^{-1} foi a melhor, a AGPR também foi a mais longa, e o valor nutricional do grupo de 50 kg t^{-1} e a IVDMD foram os mais altos. O c e o AGPR do grupo de 37.5 kg t^{-1} foram os maiores, adicionando feno de peso diferente não teve efeito significativo sobre GP_{72h} de sorgo-sudangrass, A e lag，Considerando a qualidade da fermentação da silagem e o valor nutricional da ração, o melhor modo de armazenamento misto foi adicionado 37.5 kg t^{-1} de palha de trigo a sorgo-sudangrass.

Palavras-chave: Aditivos. Subprodutos. Fermentação ruminal *in vitro.* Qualidade de silagem.

Introduction

Sorghum-sudangrass hybrids (*Sorghum bicolor* × *S. sudanense*), which are natural hybrids of sorghum (*Sorghum bicolor* (L.) Moench) and sudangrass (*Sorghum sudanense* (Piper) Stapf) have the advantageous qualities of both parents, such as high tillering ability, strong lodging resistance, high disease resistance, and high yield, and are among the most common annual warm grass forages in the world (BECK et al., 2007; JUAN, 2010; PANG; ZHANG, 2004; YUAN et al., 2011). The forage yield is greatly influenced by the season; accordingly, it is very important to regulate forage grass by processing storage sorghum-sudangrass hybrids in the summer. Hay production from sorghum-sudangrass hybrids is difficult owing to the high moisture content and thick plant stems. However, these hybrids have a high soluble carbohydrate content and are easy to ensilage (VALENZUELA; SMITH, 2002). Therefore, ensiling is the principal methods to processing and store sorghum-sudangrass hybrids. The main problem in production of sorghum-sudangrass hybrid silage is the high moisture content which leads to a bad fermentation quality of silage. Therefore, increasing studies have examined to improve fermentation quality of sorghum-sudangrass hybrids silage.

Recent research on ensiling technology for sorghum-sudangrass hybrids has focused on the regulation of silage materials (AKDENIZ et al., 2012; BI et al., 2018; GUL et al., 2008; JIANG et al., 2005) and additives (DOLEZAL, 2009; HAN et al., 2015; ZHANG et al., 2010). Chopped straw is a most effective absorbent, consequently, mixing straw with grass is an accepted technique for reducing effluent loss, increasing dry matter and improving fermentation quality of mixed silage (GALLEGOS et al., 2018). Therefore, we suppose mixing straw with sorghum-sudangrass hybrids could improving the fermentation quality of mixed silage. However, mixed silage of sorghum-sudangrass hybrids has not been well explored. In this study, the nutritional characteristics of silage materials, including the nutritive value, fermentation quality, 72h dry matter digestibility, and gas dynamics were examined *in vitro* in a simulated rumen fermentation system of sorghum-sudangrass hybrid silage mixed with various quantities and kinds of hay. The results of this study provide a scientific foundation for the production of high-quality sorghum-sudangrass hybrid silage.

Materials and Methods

Ensiling materials

Sorghum-sudangrass hybrids (*Sorghum bicolor* × *S. sudanense* Jicao 2) were planted at the Hengshui Comprehensive Research Station Forage Industrialization Technological System of PRC (37°44′N, 115°42′E, 20 m.a.s.l.) on May 15 and harvested in September 2014 (119 d) when the phenological stage of plant was in waxy ripe stage. The dry ingredients (maize straw, wheat straw, and alfalfa hay) were all provided by Hengshui Station. Sorghum-sudangrass plants were chopped using a rubbing and cutting machine, and the dry ingredients were chopped into pieces of 1-2 cm using a straw cutter. The chemical composition of ensiling materials is shown in Table 1.

Table 1. Chemical composition of ensiling materials.

Type	Dry matter content	Crude protein	Neutral detergent fiber	Acid detergent fiber	Water soluble carbohydrate
Sorghum-sudangrass hybrids	170.41	71.92	617.82	390.41	39.12
Wheat straw	921.84	37.13	726.01	464.37	9.82
Corn stalk	922.28	58.90	647.69	366.67	31.22
Alfalfa hay	902.72	165.82	475.62	332.92	24.23

Units: g kg^{-1}.

Experimental design and ensiling

The effects of the addition of dry ingredients on sorghum-sudangrass silage were examined during a 42 day fermentation period. Treatments were arranged in a 3 × 4+1 experimental design, with 3 repetitions in each treatment. The effects of 1) the dry ingredient type and 2) the quantity of dry ingredients added on sorghum-sudangrass silage were examined. Three different dry ingredients were evaluated in the ensiling process for sorghum-sudangrass as follows: 1) maize straw (MS), 2) wheat straw (WS), and 3) alfalfa hay (AH). The amounts of dry ingredients were 12.5 kg t^{-1}, 25 kg t^{-1}, 37.5 kg t^{-1}, and 50 kg t^{-1} (labeled 1, 2, 3, and 4, respectively). A control treatment of sorghum-sudangrass only was also included (CK).

After mixing, the mixture of each treatment was packed into three 22.82 L plastic pails (20 kg per pail per repetition) lined with polyethylene film bags to ensure an anaerobic environment. Silage pails were opened after a 42 day fermentation period and samples were collected from each pail.

Fermentation quality

After the pails were opened, 20 g of the silage from each pail (each repetition) was diluted with 180 mL of distilled water in a conical flask, sealed with plastic wrap, maintained at 4°C for 24 h, and then filtered through four layers of cheesecloth and a qualitative filter paper. The filtrate was further processed with a dialyzer of 0.22 μm. The pH value of the filtrate was measured using a glass electrode pH meter (pHS-3C; INESA Scientific Instrument, Shanghai, China), and then the ammonia-N (NH_3-N) content was determined following the methods of Broderick and Kang (1980) and non-protein nitrogen (NPN) was determined following Licitra et al. (1996). The organic acid contents, including lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), were determined by high-performance liquid chromatography (HPLC) (LC-10A; Shimadzu, Tokyo, Japan) and the analytical conditions were as follows: column, Shdex RSpak KC-811S-DVB gel C (8.0 mm 9 30 cm, Shimadzu); oven temperature, 50°C; mobile phase, 3 mmol L^{-1} $HClO_4$; flow rate, 1.0 mL $min^{-1;}$ injection volume 5 μL; detector, SPD-M10AVP. By analysing the rate of lactic, acetic and butyric acid separately, the fermentation quality grade of mixed silage was evaluated by the FLIEG's scores method (GUO et al., 2008).

Chemical composition

A total of 200 g of silage from each pail was dried in 65°C for about 48 h and weighed to determine the dry matter(DM). The silage samples were ground using a cyclone mill to pass a 1-mm-screen for the measurement of total N (TN), crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Kjeldahl N (i.e., TN) was analyzed following method 954.01 of the Association of Official Analytical Chemists (HELRICH, 1990). CP was calculated as Kjeldahl N × 6.25 (VAN SOEST et al., 1991), and WSC was analyzed by the methods of McDonald and Henderson (2010). An automatic fiber analyzer (Ankom 2000i full; Ankom Technology Corporation, Macedon, NY, USA) was used to analyze NDF and ADF (HELRICH, 1990).

Rumen fluid collection

Rumen fluid collection and in vitro batch cultures were performed at the State Key Laboratory of Animal Nutrition of China Agricultural University. Four rumen-cannulated lactating Holstein dairy cows (body weight = 510 ± 29 kg; days in milk = 49±8 days; daily milk yield = 16.2 ± 0.77 kg) were used as donor animals for rumen fluid collection. The animals were housed in individual tie stalls (9 m^2), each with separate water and feed bunk. The cows' daily feed was 4.0 kg alfalfa hay, 3.5 kg whole maize silage and 5.5 kg commercial concentrate consisting of 530 g maize meal/kg, 140 g soybean meal/kg, 120 g distillers dried grains/kg, 70 g cotton seed meal/kg, 40 g rape seed meal/kg, 10 g calcium hydrogen phosphate ($CaHPO_4$)/kg, 10 g sodium chloride (NaCl)/kg, 10 g limestone/kg, 10 g sodium bicarbonate/kg and 10 g trace mineral/kg and vitamin premix. The ration was divided equally into two portions and fed at 07:00 and 19:00 h, respectively. Rumen fluid, obtained from four animals 1 h before the morning feeding, was filtered through four layers of gauze and mixed in equal proportions to achieve a representative rumen fluid, held in a water-bath at 39°C in an atmosphere of carbon dioxide (CO_2) and used in the later *in vitro* batch culture. All animal care, surgical procedures, and rumen fluid collection were approved by the Institutional Animal Care and Use Committee at China Agricultural University ([2006]398).

In vitro batch cultures

The silage samples (500 mg each) were weighed and added to a total of 65 glass bottles (five bottles per treatment) with butyl rubber stoppers. Fresh buffer solution (pH 6.8) was prepared following the methods of Menke and Steingass (1988). Under a stream of nitrogen gas, each bottle was inoculated with 50 mL of the buffer solution followed by 25 mL of rumen fluids (filtered through four layers of cheesecloth). Anaerobic N_2 was purged into each bottle for 5 s to remove headspace air, and then each bottle was sealed with a butyl rubber stopper and a screw cap. All bottles were individually connected with medical plastic infusion pipes to the gas inlets of an automated trace gas recording instrument (AGRS-III, China Agricultural University, Beijing, China) to continuously record gas production (ZHANG; YANG, 2011). Three fermentations without the substrate were included as blanks. All bottles were incubated at 39°C for 72 h.

After 72 h of incubation for each bottle, the cumulative gas production volumes against incubation time were exported into a Microsoft Excel datasheet from the AGRS-III. The supernatants in each bottle were decanted and the pellets were dried at 65°C to a constant weight for determination of the residual DM. IVDMD was calculated as the DM loss, estimated as the difference between the initial and residual DM, corrected by blanks.

Calculation and statistical analysis

The cumulative gas production (GPt, mL g^{-1} DM) at time (t) was fitted to an exponential model (Eqn (1)) by an iterative regression analysis (TOMPSETT, 2000) using the nonlinear procedure of SAS for Windows as follows:

$$GPt = [1-e-c\times(t-lag)] \times A_{\circ} \qquad (1)$$

where A represents the asymptotic GP generated at a constant fractional rate (c) per unit time (h); t is the gas recording time (h), and lag represents the lag time (h) before GP commenced.

Following the methods of Garciamartinez et al. (2005), the average gas production rate (AGPR, mL/h) between the start of the incubation period and the time at which the cumulative gas production was half of its asymptotic value was calculated as follows:

$$AGPR = A \times c/(\log 2+c \times lag). \qquad (2)$$

The chemical composition results and fermentation characteristics are expressed as means ± S.E.M. Differences between groups were determined by one-way analysis of variance and post-hoc comparisons (LSD; least significant difference) using a commercial software package (SPSS version 20.0, SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P < 0.05$.

Results

Chemical composition of mixed silage of sorghum-sudangrass hybrids

As shown in Table 2, there were interactions between hay type and adding amount in DM and CP contents ($P < 0.05$). The best combination in DM and CP contents was adding 50 kg t^{-1} alfalfa hay. There was no interaction between hay type and adding amount in NDF, ADF and WSC contents ($P > 0.05$).

With respect to hay type, there were no differences in DM, ADF and WSC contents among the three dry ingredients groups ($P > 0.05$). The CP content was significantly higher for sorghum-sudangrass hybrid silage mixed with alfalfa hay (AH) than mixed with maize stalk (MS) or wheat straw (WS) ($P < 0.05$). The NDF content was significantly higher in the CS group than in the AH and WS groups ($P < 0.05$). These results indicated that the nutrient content of mixed silage was highest for alfalfa hay, followed closely by wheat straw.

Table 2. Analysis of nutritional components of sorghum-sudangrass hybrid silage.

Hay type	Amount	DM (g kg^{-1})	CP (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	WSC (g kg^{-1} DM)
Corn stalk	12.5 kg t^{-1}	192.21±2.31^{ab}	60.71±4.78^{h}	660.42±15.14	420.89±17.74	9.93±1.52
Corn stalk	25 kg t^{-1}	188.73±5.03^{abc}	62.92±3.12^{gh}	636.29±6.22	410.41±8.73	8.52±1.51
Corn stalk	37.5 kg t^{-1}	192.01±2.78^{ab}	70.22±2.71^{efg}	633.72±3.41	398.59±1.41	8.91±1.19
Corn stalk	50 kg t^{-1}	196.82±11.01^{a}	74.82±17.78^{def}	609.33±13.21	391.44±5.61	10.61±1.51
Wheat straw	12.5 kg t^{-1}	179.12±7.43^{c}	79.71±1.71^{cd}	629.42±9.03	409.73±4.72	9.42±0.32
Wheat straw	25 kg t^{-1}	185.42±2.79^{bc}	68.33±1.49^{fgh}	620.52±12.51	413.32±8.01	10.81±0.92
Wheat straw	37.5 kg t^{-1}	188.32±11.82^{abc}	66.23±5.52^{gh}	605.92±11.63	408.12±0.91	10.79±2.52
Wheat straw	50 kg t^{-1}	191.12±9.61^{ab}	67.31±3.03^{fgh}	591.83±5.14	404.22±9.93	10.44±2.61
Alfalfa hay	12.5 kg t^{-1}	161.8±6.33^{d}	77.49±6.31^{cde}	616.58±13.78	403.41±8.29	8.72±1.13
Alfalfa hay	25 kg t^{-1}	191.31±5.97^{ab}	82.81±6.08^{bc}	613.08±2.69	407.44±2.79	11.53±2.61
Alfalfa hay	37.5 kg t^{-1}	189.62±3.62^{abc}	89.22±3.29^{ab}	563.62±8.56	391.01±6.02	10.89±1.41
Alfalfa hay	50 kg t^{-1}	199.11±4.89^{a}	94.59±6.18^{a}	573.52±13.22	400.92±10.21	10.91±0.12
CK		140.61±1.82^{d}	68.12±9.31^{fgh}	613.83±24.64	401.44±3.12	10.33±1.12
Major effect analysis						
Hay type	Amount					
Corn stalk		192.42 ± 6.21	67.12 ± 6.51^{b}	635.02 ± 21.02^{a}	405.33 ± 14.67	9.51 ± 1.57
Wheat straw		186.01± 8.67	70.02 ± 5.72^{b}	611.92 ± 17.21^{b}	408.81 ± 6.73	10.44 ± 1.71
Alfalfa hay		185.52 ± 15.39	86.01 ± 7.89^{a}	591.72 ± 26.11^{c}	400.71 ± 8.89	10.55 ± 1.83
	12.5 kg t^{-1}	177.72 ± 14.12^{b}	71.90 ± 9.42^{b}	635.54 ± 22.52^{a}	411.32 ± 12.71^{a}	9.42 ± 1.15
	25 kg t^{-1}	188.52 ± 4.89^{a}	71.28 ± 9.11^{b}	623.31 ± 12.49^{a}	410.42 ± 6.64^{a}	10.36 ± 2.16
	37.5 kg t^{-1}	190.01 ± 6.52^{a}	75.22 ± 11.23^{ab}	601.13 ± 31.51^{b}	399.25 ± 8.02^{b}	10.22 ± 1.85
	50 kg t^{-1}	195.67 ± 8.52^{a}	78.89 ± 12.73^{a}	591.53 ± 18.32^{b}	398.82 ± 9.61^{b}	10.62 ± 1.51
P-Value						
Hay type		0.100	< 0.001	< 0.001	0.092	0.261
Amount		0.001	< 0.001	< 0.001	0.004	0.414
Interraction effect		0.003	< 0.001	0.061	0.082	0.366

†Different lowercase letters in the same row indicate significant differences ($P < 0.05$). DM= Dry matter, CP= Crude protein, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, WSC= Water soluble carbohydrate, CK= Control check.

With respect to the amount, the DM and CP content of mixed silage increased and the NDF and ADF content decreased as the amount of dry ingredients increased. The DM content in the 12.5 kg t^{-1} group was significantly lower than those of the other three groups ($P < 0.05$). The CP content in the 50 kg t^{-1} group was significantly higher than those in the 12.5 kg t^{-1} and 25 kg t^{-1} groups ($P < 0.05$). The NDF and ADF contents of the 50 kg t^{-1} group were significantly lower than those for the 12.5 kg t^{-1} and 25 kg t^{-1} groups ($P < 0.05$). There were no differences in WSC content among the four groups. The nutrient contents in the 37.5 kg t^{-1} and 50 kg t^{-1} groups were significantly higher than those in the 12.5 kg t^{-1} and 25 kg t^{-1} groups ($P < 0.05$), and there was no difference between the 37.5 kg t^{-1} and 50 kg t^{-1} groups.

Fermentation quality of mixed silage of sorghum-sudangrass hybrids

As shown in Table 3, there was no interaction between hay type and adding amount in pH, AN/TN, LA, PA, AA and BA contents ($P > 0.05$).

With respect to hay type, there were no differences in PA and BA contents among the three dry ingredients groups ($P > 0.05$). The LA content of the wheat straw group was significantly higher than that in the corn stalk group ($P < 0.05$). The pH values and AA content in the wheat straw group were significantly lower than those in the corn stalk group ($P < 0.05$). The AN/TN content of the corn stalk group was significantly higher than those of the other two groups ($P < 0.05$). Overall, the fermentation quality of mixed silage was best for wheat straw and worst for corn stalk.

In the terms the amount added, the AA content of mixed silage decreased as the amount of dry ingredients increased; it was significantly higher in the 12.5 kg t^{-1} group than in the 37.5 kg t^{-1} and 50 kg t^{-1} groups ($P < 0.05$). The LA content was significantly higher in this group than in the 12.5 kg t^{-1} group ($P < 0.05$). The pH value in the 12.5 kg t^{-1} group was significantly higher than those in the other three groups ($P < 0.05$). There were no differences in PA, BA, and AN/TN contents among the four groups ($P > 0.05$), but BA was not detected in the 25 kg t^{-1} and 37.5 kg t^{-1} groups, which had a better performance. Therefore, sorghum-sudangrass hybrid silage supplemented with 25 kg t^{-1} dry ingredients had the best performance with respect to fermentation quality, followed closely by the 37.5 kg t^{-1} group. The worst performed was observed for the 12.5 kg t^{-1} group.

As summarized in Table 4, the FLIEG's scores for separate silage of sorghum-sudangrass hybrids were only classified as "Fair," indicating bad performance. All other FLIEG's evaluations for treatment groups were classified as "Excellent" or "Well."

Table 3. Analysis of the fermentation quality of sorghum-sudangrass hybrid silage.

Hay type	Amount	pH	AN/TN* ($g\ kg^{-1}$)	Lactic acid ($g\ kg^{-1}$ DM)	Acetic acid ($g\ kg^{-1}$ DM)	Propionate acid ($g\ kg^{-1}$ DM)	Butyric acid ($g\ kg^{-1}$ DM)
Corn stalk	12.5 $kg\ t^{-1}$	3.84±0.10	40.13±8.28	54.51±6.81	28.91±1.72	44.23±0.91	0±0.00
Corn stalk	25 $kg\ t^{-1}$	3.73±0.04	44.07±13.61	73.22±10.68	29.71±2.47	47.33±2.61	0±0.00
Corn stalk	37.5 $kg\ t^{-1}$	3.72±0.10	36.32±4.89	66.42±3.52	26.71±3.12	50.92±2.92	0±0.00
Corn stalk	50 $kg\ t^{-1}$	3.70±0.04	31.44±6.89	67.53±6.33	24.42±3.72	48.65±3.33	0±0.00
Wheat straw	12.5 $kg\ t^{-1}$	3.69±0.04	28.74±11.82	64.02±27.91	25.53±8.41	51.49±17.02	1.03±0.11
Wheat straw	25 $kg\ t^{-1}$	3.68±0.01	33.93±1.72	94.82±9.42	16.61±7.72	50.44±14.84	0±0.00
Wheat straw	37.5 $kg\ t^{-1}$	3.69±0.07	28.21±14.23	80.33±12.55	16.63±7.42	40.42±13.12	0±0.00
Wheat straw	50 $kg\ t^{-1}$	3.66±0.05	28.67±4.52	79.72±16.11	13.01±4.24	32.94±6.92	1.49±0.52
Alfalfa hay	12.5 $kg\ t^{-1}$	3.75±0.03	28.44±15.12	56.56±12.42	23.21±10.33	49.82±18.04	1.00±0.31
Alfalfa hay	25 $kg\ t^{-1}$	3.69±0.08	21.22±4.79	74.12±21.81	23.02±4.81	47.21±9.55	0±0.00
Alfalfa hay	37.5 $kg\ t^{-1}$	3.70±0.06	28.62±6.01	87.21±19.02	15.92±2.91	44.22±16.88	0±0.00
Alfalfa hay	50 $kg\ t^{-1}$	3.72±0.04	22.11±1.24	77.49±12.02	14.51±0.72	40.32±9.18	0±0.00
CK		3.63±0.06	29.31±1.92	98.62±6.31	31.61±11.42	69.54±4.55	5.01±1.00
Major effect analysis							
Hay type	Amount						
Corn stalk		3.75 ± 0.09[a]	38.02 ± 9.11[a]	65.39 ± 9.42[b]	27.43 ± 3.21[a]	47.82 ± 3.41	0.00 ± 0.00
Wheat straw		3.68 ± 0.04[b]	29.89 ± 8.52[b]	79.72 ± 19.11[a]	17.93 ± 8.92[b]	43.81 ± 14.02	0.62 ± 0.12
Alfalfa hay		3.71 ± 0.05[ab]	25.12 ± 8.11[b]	73.78 ± 18.44[ab]	19.12 ± 6.53[b]	45.54 ± 12.51	0.31 ± 0.11
	12.5 $kg\ t^{-1}$	3.76 ± 0.09[a]	32.52 ± 12.03	58.44 ± 16.22[b]	25.78 ± 7.22[a]	48.52 ± 12.83	0.72 ± 0.12
	25 $kg\ t^{-1}$	3.70 ± 0.05[b]	33.02 ± 12.32	80.84 ± 16.78[a]	23.13 ± 9.04[ab]	48.33 ± 9.01	0.00 ± 0.00
	37.5 $kg\ t^{-1}$	3.69 ± 0.07[b]	31.03 ± 0.92	78.01 ± 14.76[a]	19.74 ± 6.81[b]	45.22 ± 11.71	0.00 ± 0.00
	50 $kg\ t^{-1}$	3.69 ± 0.05[b]	27.43 ± 5.92	74.89 ± 12.01[a]	17.33 ± 6.12[b]	40.65 ± 9.12	0.52 ± 0.11
P-Value							
Hay type		0.033	< 0.001	< 0.001	< 0.001	0.187	0.635
Amount		0.040	0.202	< 0.001	< 0.001	0.241	0.628
Interraction effect		0.590	0.208	0.204	0.206	0.297	0.907

*AN/TN= The proportion of NH_3-N to total nitrogen.
†Different lowercase letters in the same row indicate significant differences ($P < 0.05$), CK= Control check.

Table 4. FLIEG's scores of silage.

Groups	Lactic acid score	Acetic acid score	Butyric acid score	Total score	Rank
MS1	9	16	50	75	Well
MS2	12	18	50	80	Well
MS3	11	18	50	79	Well
MS4	12	19	50	81	Excellent
WS1	10	18	50	78	Well
WS2	17	20	50	87	Excellent
WS3	17	20	50	87	Excellent
WS4	19	20	50	89	Excellent
AH1	10	19	50	79	Well
AH2	13	19	50	82	Excellent
AH3	17	20	50	87	Excellent
AH4	17	20	50	87	Excellent
CK	12	18	30	60	Fair

†1) MS, WS and AH correspond to maize straw, wheat straw, and alfalfa hay, respectively. The number 1, 2, 3 and 4 correspond to the adding amount of 12.5 kg t^{-1}, 25 kg t^{-1}, 37.5 kg t^{-1}, and 50 kg t^{-1}, respectively, CK= Control check.

IVDMD, kinetic parameters of gas production of sorghum-sudangrass hybrid silage

As shown in Table 5, there was no interaction between hay type and adding amount in IVDMD, GP_{72h}, A, C, Lag, Half time and AGPR contents (P > 0.05).

With respect to hay type, the IVDMD, C, and AGPR of the AH group were significantly higher than in the other two groups (P < 0.05). The half time of the AH group was significantly shorter than in the other two groups (P < 0.05). There were no differences in GP_{72h} , A, and lag among the three groups (P > 0.05).

In terms of the amount added, the IVDMD content was significantly higher in the 50 kg t^{-1} group than in the 12.5 kg t^{-1} group (P < 0.05). The C and AGPR contents of the 37.5 kg t^{-1} group were significantly higher than those of the 25 kg t^{-1} groups (P < 0.05). The half time of the 25 kg t^{-1} group was significantly longer than those of the 12.5 kg t^{-1} and 37.5 kg t^{-1} groups (P < 0.05). There were no differences in GP_{72h}, A, and lag among the four groups (P > 0.05).

Table 5. Analysis of IVDMD and kinetic parameters of gas production for sorghum-sudangrass hybrid silage.

Hay type	Amount	IVDMD ($g\ kg^{-1}$)	GP_{72h} ($mL\ g^{-1}$)	A (mL)	C ($mL\ h^{-1}$)	Lag (h)	Half time (h)	AGPR ($mL\ h^{-1}$)
Corn stalk	12.5 $kg\ t^{-1}$	596.41±51.33	78.69±1.73	78.69±1.73	0.22±0.02	0±0.00	2.21±0.10	25.11±2.92
Corn stalk	25 $kg\ t^{-1}$	632.33±49.22	80.28±3.61	80.28±3.61	0.21±0.01	0±0.00	2.26±0.07	24.08±1.15
Corn stalk	37.5 $kg\ t^{-1}$	614.89±45.33	80.23±4.85	80.23±4.85	0.22±0.01	0±0.00	2.23±0.15	24.97±2.25
Corn stalk	50 $kg\ t^{-1}$	660.59±19.72	83.25±2.40	83.25±2.40	0.23±0.02	0±0.00	2.17±0.06	27.47±1.69
Wheat straw	12.5 $kg\ t^{-1}$	645.01±33.62	83.05±2.50	83.05±2.50	0.23±0.01	0±0.00	2.18±0.05	27.11±0.55
Wheat straw	25 $kg\ t^{-1}$	657.92±19.63	81.86±4.49	81.86±4.49	0.19±0.03	0±0.00	2.35±0.12	22.67±2.47
Wheat straw	37.5 $kg\ t^{-1}$	651.01±5.12	79.80±3.21	79.80±3.21	0.23±0.03	0±0.00	2.17±0.12	26.34±2.02
Wheat straw	50 $kg\ t^{-1}$	654.56±20.16	85.55±3.56	85.55±3.56	0.21±0.03	0±0.00	2.28±0.15	25.92±3.60
Alfalfa hay	12.5 $kg\ t^{-1}$	629.93±84.61	76.77±7.37	76.77±7.37	0.25±0.01	0±0.00	2.06±0.02	28.26±3.07
Alfalfa hay	25 $kg\ t^{-1}$	661.42±27.01	82.22±4.28	82.22±4.28	0.22±0.02	0±0.00	2.23±0.10	25.51±1.77
Alfalfa hay	37.5 $kg\ t^{-1}$	684.81±26.02	79.89±3.93	79.89±3.93	0.31±0.05	0±0.00	1.88±0.17	24.08±5.03
Alfalfa hay	50 $kg\ t^{-1}$	673.53±30.11	83.08±2.35	83.08±2.35	0.31±0.05	0±0.00	1.91±0.15	24.97±4.84
CK		646.63±23.81	82.61±4.80	82.61±4.80	0.25±0.03	0±0.00	2.12±0.12	29.05±3.18
Major effect analysis								
Hay type	Amount							
Corn stalk		626.02±44.41^{b}	81.68±5.82	81.68±5.82	0.23±0.03^{b}	0.00±0.00	2.20±0.15^{a}	26.32±3.19^{b}
Wheat straw		652.12±46.67^{b}	82.61±6.39	82.61±6.39	0.22±0.05^{b}	0.00±0.00	2.24±0.22^{a}	25.70±4.76^{b}
Alfalfa hay		662.41±19.42^{a}	81.44±7.49	81.44±7.49	0.28±0.07^{a}	0.00±0.00	1.99±0.25^{b}	32.70±8.14^{a}
	12.5 $kg\ t^{-1}$	623.78±56.55^{b}	79.04±7.74	79.04±7.74	0.27±0.02^{ab}	0.00±0.00	2.04±0.10^{b}	30.32±3.34^{a}
	25 $kg\ t^{-1}$	650.21±32.67^{ab}	80.76±6.64	80.76±6.64	0.23±0.04^{b}	0.00±0.00	2.20±0.17^{a}	26.74±3.22^{b}
	37.5 $kg\ t^{-1}$	650.51±40.11^{ab}	79.38±5.69	79.38±5.69	0.28±0.07^{a}	0.00±0.00	2.02±0.25^{b}	31.48±6.89^{a}
	50 $kg\ t^{-1}$	662.81±28.23^{a}	83.96±5.58	83.96±5.58	0.25±0.09^{ab}	0.00±0.00	2.12±0.31^{ab}	29.87±9.01^{ab}
P-Value								
Hay type		0.028	0.325	0.544	0.011	0.922	0.001	< 0.001
Amount		0.030	0.202	0.635	0.014	0.915	0.041	0.034
Interraction effect		0.715	0.496	0.317	0.206	0.917	0.622	0.306

†Different lowercase letters in the same row indicate significant differences ($P < 0.05$). IVDMD= *in vitro* dry matter digestibility, GP_{72h}= *the cumulative gas production* at 72 h, *A= the asymptotic gas production,* C= *constant fractional rate,* AGPR= *the average gas production rate,* CK= Control check.

Discussion

Separate silage of sorghum-sudangrass hybrids

The moisture content of sorghum-sudangrass hybrids is very high, providing a favorable environment for the proliferation of *Clostridium* spp. (WEI; WANG, 2010). Butyric acid secreted by *Clostridium* spp. has a bad odor and decreases the fermentation quality of silage (BORBA et al., 2012). In the present study, the *FLIEG's score* evaluation ranking for separate silage was only "Fair" owing to a higher content of butyric acid, which means that sorghum-sudangrass hybrids could be ensiled alone, but the fermentation quality was not good. This result was consistent with those of Ji et al. (2012). All mixed silage of sorghum-sudangrass hybrids had a good fermentation quality.

Chemical composition and fermentation quality of mixed silage

The proportion of NH_3-N to TN, and LA and VFA contents are important indicators of the fermentation quality of silage (SELMEROLSEN, 2010). The NH_3-N/TN content not only reflects the degree of proteolysis during silage fermentation, but also affects the utilization efficiency of N in the rumen (TUULOS et al., 2015). Higher NH_3-N production in silage is associated with a lower silage fermentation quality. In this study, the NH_3-N/TN content of sorghum-sudangrass hybrid silage was lower when mixed with AH or WS than MS. This may be explained by the low pH value of the silage mixed with AH or WS. A low pH can inhibit the protease activity and thus decrease the NH_3-N/TN content (CUSSEN et al., 2010). The NH_3-N/TN, AA, and PA contents of mixed silage gradually decreased as the amount of dry ingredients increased. It is possible that the DM content and nutritive value of sorghum-sudangrass hybrid silage were significantly improved after mixing with dry ingredients, which could promote the activity of LAB and lignocellulose degradation enzymes to improve the fermentation quality (TIAN et al., 2015).

IVDMD, kinetic parameters of gas production of mixed silage

Fodder degradation in the rumen is the consequence of the decomposition and utilization of nutrients in fodder by rumen microorganisms (ZHANG et al., 2013). In the present study, the IVDMD content of mixed silage decreased according to AH group > WS group > MS group and 50 kg t^{-1} > 37.5 kg t^{-1} > 25 kg t^{-1} > 12.5 kg t^{-1}. There are two potential explanations for the high IVDMD content of silage mixed with AH. First, alfalfa is a legume, and sorghum-sudangrass hybrids, maize, and wheat are graminaceous crops. From the perspective of complementary functions of nutrients, those in the combination of grass and legume forages are more favorable than those for a single kind of forage (YANG et al., 2008). The sorghum-sudangrass hybrid silage mixed with AH had balanced nutrients, which can accelerate the growth of rumen microorganisms and improve the fermentation potential of protein and water-soluble carbohydrates (PRASAD et al., 2010), thereby improving the IVDMD content. Similar results have been reported in a study by Cui et al. (2012). In addition, the low ADF content of AH may explain the high IVDMD content.

Carbohydrates are the main source of *in vitro* gas production of forages. Although protein can also produce gas during fermentation, it is not comparable in volume to carbohydrate fermentation (CONE; AHVAN, 1999). In the present study, there were no differences in GP_{72h} and A contents of mixed silage among the three dry ingredient groups. This can probably be attributed to the inferior fermentation gas contribution of protein relative to carbohydrates. The C and AGPR of silage mixed with AH were highest among the three dry ingredients, possibly reflecting the high contents of cellulose, hemicellulose, and lignin in wheat straw and maize straw, reducing the gas generation rate of the fermentation substrate. The C and AGPR contents of mixed silage in the 37.5 kg t^{-1} group were highest and significantly higher than those in the 25 kg t^{-1} group, suggesting that adding 37.5 kg t^{-1} AH facilitated the balance of energy and protein in the fermentation substrate.

The lag is significantly positively correlated with the NDF and ADF contents and significantly negatively correlated with the CP and neutral detergent soluble (NDS) contents of the fermentation substrate (SUN et al., 2014). In the present study, the lag was zero in all treatments; it is possible that the very high content of WSC in sorghum-sudangrass hybrids provides ample nutrients for rapid degradation by rumen microorganisms.

Conclusions

Sorghum-sudangrass hybrids can be ensiled alone, but have a high butyric acid content and a low fermentation quality. The addition of various hay types and amounts could significantly improve the fermentation quality of mixed silage. Synthetically considering the nutritive value and fermentation quality, 50 kg t^{-1} alfalfa hay had the highest IVDMD content, 37.5 kg t^{-1} alfalfa hay had the highest gas production rate, and 50 kg t^{-1} wheat straw had the highest GP_{72h} and A, the best mixed silage mode was the addition of 37.5 kg t^{-1} wheat straw. These results indicate that the addition of 37.5 kg t^{-1} wheat straw could not only improve the fermentation quality of Sorghum-sudangrass hybrids silage, but also maximize the crop value and minimize agricultural losses.

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