Zhang SJ, Chaudhry AS, Osman A, Shi CQ, Edwards GR, Dewhurst RJ, Cheng L. Associative effects of ensiling mixtures of sweet sorghum and alfalfa on nutritive value, fermentation and methane characteristics. *Animal Feed Science and Technology* 2015, 206, 29-38.

DOI link to article:

http://dx.doi.org/10.1016/j.anifeedsci.2015.05.006

Date deposited:

10/02/2016

Embargo release date:

21 May 2016

Associative effects of ensiling mixtures of sweet sorghum and alfalfa on nutritive value, fermentation and methane characteristics

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ABSTRACT

Combining sweet sorghum (**SS**) with alfalfa (**AF**) for ensiling has the potential to improve the nutritive value and fermentation characteristics of resultant silages. However, the optimal combination and the associative effects of SS and AF for ensilage have not been studied. Therefore, the aim of this study was to determine the fermentation characteristic and nutritive value of silage mixtures with six different SS to AF ratios. The two forages were ensiled in air free silos for 150 days at room temperature as mixtures containing 0: 100, 20: 80, 40: 60, 60: 40, 80: 20, and 100: 0 of SS : AF on a fresh weight basis. As the proportion of SS increased in silage, the content of ash, crude protein, saponins, ammonia, acetic acid, propionic acid and pH decreased, while neutral detergent fiber, acid detergent fiber in organic matter, acid detergent lignin, water-soluble carbohydrate, starch, total phenolics and condensed tannins content increased. The silages were evaluated in 24-hour incubations with rumen liquor. The *in-vitro* rumen degradability of dry matter and organic matter as well as gas production, pH, ammonia, total volatile fatty acids and methane decreased as the proportion of SS increased in the silage mixtures. This study suggests that high quality silages can be made with SS: AF ratios of 20:80 and 40:60. These silage mixtures offer an opportunity to optimize the nutrient supply for ruminant production.

Keywords: Tannins; Saponins; *In-vitro* methane production; Volatile fatty acids; Gas production; pH

Abbreviations: **SS**, sweet sorghum; **AF**, alfalfa; **DM**, dry matter; **OM**, organic matter; **CP**, crude protein; **aNDFom**, neutral detergent fibre in OM; **ADFom**, acid detergent fibre in OM; **ADL**, acid detergent lignin; **EE**, ether extract; **IVDMD**, *in-vitro* DM degradability; **IVOMD**, *in-vitro*

OM degradability; **tVFA**, total volatile fatty acids; **WSC**, water-soluble carbohydrate; **GP**, gas production; **CH_4**, methane; **NH_3**, ammonia; **SP**, saponins; **TP**, total phenolics; **CT**, condensed tannins.

1. Introduction

Sweet sorghum (*Sorghum bicolor,* **SS**) is a promising forage in the arid, semi-arid and high salinity areas due to its rapid growth, high biomass yield (Qu et al., 2014), drought tolerance and high water-use efficiency (Wu et al., 2010). Sweet sorghum can be conserved as ruminant feed through ensilage (Calabrò et al., 2010a). However, the crude protein (**CP**) content in SS fresh and SS silage (~ 100 g CP/kg DM; Colombini et al., 2012) is insufficient to fulfil the requirement of growing or lactating ruminants (NRC, 2007). In order to meet the CP requirement of ruminants, forages with a high CP content, such as legume, can be mixed with low CP forages before or after ensiling. However, silage only making from legume is often challenging, due to its low water-soluble carbohydrates (**WSC**) content and high buffering capacity (Fisher and Burns, 1987) and extensive proteolysis during ensiling (McDonald et al. 1991). Ozturk et al. (2006) showed that ensiling maize with alfalfa (*Medicago sativa,* **AF**) is a feasible strategy to increase the CP content and improve the nutritive value of silage. Differently to temperate areas,, maize production is low in the arid and high salinity areas around the world (Qu et al., 2014), and SS is an attractive alternative in these regions (Wu et al., 2010). There have been few studies to provide detailed investigation of the feasibility of mixing SS and legume forages for silage making. As a widely grown perennial legume with a deep root system and strong resistance to drought, AF can be grown well in arid, semi-arid areas. Therefore, AF was selected as a candidate legume for this study as bases for developing optimal silage mixtures for animal production in arid, semi-arid regions. The aim of this study was to investigate the associative effects of ensiling mixtures of SS and AF on nutritive value and fermentation characteristics of resulting silages. It tested the hypothesis that synergies from combining the two forages mean that the nutritive value and fermentation characteristics of mixed silages are better than would be predicted from values for silages prepared from the single forages.

2. Materials and methods

2.1 Forage harvesting and silage making

The cultivars used for SS and AF in this study were *Cowley* with 22.5% Brix value and *Hetian Big-leaf* respectively. Both SS and AF were sown at the Agricultural Research Station of Tarim University, XinJiang, China. Whole plant of SS and AF were harvested at milky stage and at early bloom stage (10% flowering rate), respectively, using a grass hook and leaving a stubble of 5 cm. Forage sample was chopped into 2.5 cm particle size by a multi-function chopper (9DF53, Yanbei Animal Husbandry Machinery Group Co. Ltd., Beijing, China). About 500 g sample of each fresh forage of SS and AF was stored directly at -20°C until analysed for proximate composition. Plastic silos were used to make chopped forages into six silage types, with different SS to AF ratios (containing 0, 20, 40, 60, 80 and 100% SS based on fresh weight). The fresh weight of forages in each silo was 1.5 kg and ten replicates of each silage type were made. The forage mixtures were manually compressed to remove air before the silos were screw capped. The silos were stored in the dark at room temperature.

2.2 Quality analysis of silage

2.2.1 Chemical analysis

To mimic the silage based livestock production system in arid and semi-arid regions in the world, where silages are normally made in summer and fed out in autumn and winter when feed supply is low; the silos were opened 150 days post ensiling and a 500 g fresh weight sample was collected per silo for analysis. A 15 g fresh weight sample was blended with 135 mL distilled water for 1 min followed by filtration through two layers of cheesecloth. The supernatant was then tested for pH using pH meter (pH209, Hanna Instuments., Edge, USA). Two 15 mL subsamples of the extract were centrifuged at 2500 rpm for 10 min at 4 °C (MSE Mistral 3000, Sanyo Gallenkamp, Leicestershire, UK) , and then acid extraction (Chaudhry and Khan, 2012) was performed on supernatant before ammonia (**NH_3**) and organic acids analysis. The concentration of NH_3 was analyzed by Pentra 400 (Horriba Ltd, Kyoto, Japan) according to the method described by Rhine et al. (1998). Lactic, acetic and propionic acids were determined using GC (Shimadzu Ltd, Kyoto, Japan) according to Fussell and McCalley (1987).

Subsamples of 500 g per silage type and fresh forage of SS and AF prior to ensiling were dried at 65 °C in an oven and then ground through a 1 mm sieve using a mill (Christy and Norris Co. Ltd., Suffolk, UK), and analysed in triplicate for dry matter (**DM**), ash, ether extract (**EE**) according to AOAC (2005) procedures. Ash-free neutral detergent fiber in organic matter with addition of α-amylase (**aNDFom**), ash-free acid detergent fiber in organic matter (**ADFom**) and acid detergent lignin (**ADL**) were determined according to the methods of Van Soest (1991). Crude protein (**CP**) was calculated by multiplying 6.25 with the content of nitrogen (**N**), which was determined using an Elementar Vario Macro Cube (Elementar, Hanau, Germany). WSC were determined by Spectrophotometer (Libra S11, Biochrome, Cambridge, UK) following the method of Koehler (1952). The starch was tested by the method of Kent-Jones and Amos (1967) as described by Chaudhry and Khan (2012). Total phenolics (**TP**) of silage samples were measured using the Folin–Ciocalteu method (Singleton and Rossi, 1965). Total condensed tannins (**CT**) and saponins (**SP**) of silage samples were measured according to the method described by Osman (2004) and Khan and Chaudhry (2010), respectively.

2.2.2 Mineral Analysis

The concentrations of Ca, P, K, Mg, Fe, Zn, Cu, Na, Mn, Mo and Co from each silage type were determined, in triplicate, using a VISTA-MPX CCD simultaneous ICP-OES (Varian Inc., Melbourne, Australia). The samples and the standard solutions for mineral analysis were prepared according to the methods of Chaudhry and Jabeen (2011) and Ramdani et al. (2013).

2.3 Measurement of in-vitro fermentation parameters

2.3.1 Preparation of rumen fluids and buffered inoculums

Six Texel × Mule castrated lambs (45 ± 1.2 kg live weight) were fed on nutritionally balanced perennial ryegrass-concentrate diet prior to slaughter at an abattoir (Linden Food, UK). The lambs were slaughtered under The Welfare of Animals at the Time of Killing (WATOK) Regulations of the UK (DEFRA, 2013). Rumen samples were collected immediately post slaughtering. The rumen fluid was harvested by filtering through double layers of cheesecloth into pre-warmed (39 °C) thermo bottles and immediately transported to the laboratory. The rumen fluid was poured into a pre-warmed brown bottle containing artificial saliva (McDougall, 1948) to prepare buffered inoculum. This buffered inoculum was kept anaerobic by flushing it with anaerobic grade CO_2 before aliquots were added using a dispenser pump, and bottles closed (Chaudhry and Mohamed, 2011).

2.3.2 In-vitro incubations

A total of 200 mg of each type of dried silage in four replicates were separately weighed into 50 mL graduated glass syringes (KR Analytical Ltd., Sanitex, UK) fitted with plungers. A mixture of ruminal fluid and buffer (20 mL) was dispensed into each syringe before its incubation in a shaking water bath (Grant Instruments, Cambridge, UK) at 39 °C for 24 h. At the same time, incubations without any silage sample of three empty syringes served as the blanks to correct the final values of respective degradability, gas production (**GP**) and other fermentation parameters. The volume of GP in each syringe was recorded at 2, 4, 6, 8, 10, 20, 22 and 24 h of incubation.

2.3.3 Determination of pH, ammonia, in-vitro dry matter and organic matter degradability

Fermentation in the syringes was terminated at 24 h by transferring the syringes from the water bath to an ice-filled container. About 15 mL of headspace gas in each syringe was transferred into a vacuum tube through a three-way valve (Fisher Scientific, Loughborough, UK) for methane (**CH_4**) analysis. Each incubated sample was tested for pH and then centrifuged at 2500 rpm for 10 min at 4 °C (MSE Mistral 3000, Sanyo Gallenkamp., Leicestershire, UK). A total of 2 mL of the supernatant from each centrifuge tube was used for later volatile fatty acid (**VFA**) analysis. An additional 2 mL of the supernatant from each sample was used for NH_3 analysis. The remaining supernatant, along with all residues in each centrifugal tube were dried at 65°C and weighed for *in-vitro* DM degradability (**IVDMD**). The dried residues were decanted into crucibles and ashed at 550°C for measuring *in-vitro* organic matter degradability (**IVOMD**).

2.3.4 Ammonia, volatile fatty acid and methane analysis

NH_3 was analysed by Pentra 400 (Horriba Ltd., Kyoto, Japan) with a calibrated standard of NH_3-N according to Rhine *et al*. (1998). Volatile fatty acids concentration along with relevant standards (Sigma Aldrich, Gillingham UK) was analyzed by a GC (Shimadzu., Kyoto, Japan) as described by Eun and Beauchemin (2007). Total VFA concentration (mM) was determined by summing the areas of individual VFA in each sample and each VFA were expressed as % of total VFA. The CH_4 analysis was performed on a Fisons 8060 GC using a split injection linked to a Fisons MD800 MS as described by Bhatta et al. (2009).

2.4 Mathematical calculations and Statistical analysis

The GP data for each silage mixture were fitted to the exponential model $Y= a+b\ (1-e^{-ct})$ as described by Ørskov and McDonald (1979) using the Curve Fit software for the estimated parameters. Where a = instant GP from rapidly soluble fraction, b = slow GP from insoluble fraction, c = the rate of GP from slowly insoluble fraction (b), t = incubation time and Y = GP at time t. The SPSS statistical package (SPSS Inc., Chicago, USA) was used for statistical analysis of all the parameters. One-way ANOVA was used to examine the linear and quadratic effect of silage types on chemical composition, mineral profile, GP, GP parameters (a, b and c), IVDMD, IVOMD, CH_4, pH, NH_3 and VFA adopting a significance level of $P<0.05$. The statistical model included silage type as treatment effect. Tukey post-hoc was used for multiple comparisons of means across the monocultures or the mixtures with different ratios of SS and AF. Treatment differences were considered to be significant when $P < 0.05$.

3. Results

3.1 Chemical composition of AF and SS prior to ensiling

The chemical composition of AF and SS forages is presented in Table 1. AF was significantly ($P<0.001$) higher in DM, Ash, CP and EE than SS, whereas SS was significantly ($P<0.05$) higher in WSC, Starch, aNDFom, ADFom and ADL than AF.

3.2 Chemical composition of SS-AF silage mixtures

The chemical composition of the silages is given in Table 2. The concentrations of DM, Ash, CP, EE, SP in the SS-AF silage mixture significantly ($P<0.05$) decreased, whereas aNDFom, ADFom, ADL, WSC, starch, TP and CT significantly ($P<0.05$) increased as the proportion of SS increased in the silage. The CP and WSC content in 0% SS silage was 3.6 times higher and 4.4 times lower than in 100% SS silage, respectively (Table 2). The ash content in 0% SS silage (116 g/kgDM) was about 50% higher than that of 100% SS silage (i.e., 100 % SS silage; 73 g/kg).

3.3 Fermentation characteristics of SS-AF silage mixtures

The fermentation characteristics of the silage mixtures are shown in Table 3. The pH, NH_3, acetic acid and propionic acid content significantly ($P<0.05$) decreased, while lactic acid content significantly ($P<0.001$) increased as the proportion of SS in the silage mixtures increased from 0% to 100%.

3.4 Mineral profile of SS-AF silage mixtures

Mineral profile of the silage mixtures are presented in Table 4. The content of K, Ca, P, Mg, Na, Fe and Zn significantly ($P<0.001$) decreased as more SS was included in the silage mixtures. No significant ($P>0.05$) differences in the content of Mn, Cu, Mo and Co were observed in the silage mixtures.

3.5 In-vitro fermentation profiles of SS-AF silage mixtures

The pH, NH_3, IVDMD, IVOMD, tVFA and individual VFA except butyrate decreased as the proportion of SS in silage mixtures was significantly ($P<0.05$) increased. IVDMD and IVOMD in the silage mixtures with SS at 0%, 20% and 40% inclusion were significantly (*P*<0.05) higher than those with SS at 80% and 100% level (Table 5).

3.5 In-vitro gas production, kinetic parameters and methane of SS-AF silage mixtures

Methane, GP and values for GP kinetics model of in-vitro fermentation are given in Table 6. *In-vitro* cumulative GP between 2 and 24 h of incubation differed among the silage types. The AF silage and the silage mixtures containing 20% and 40% of SS produced more gas than the other silage mixtures. The silage made with 100% SS had the significantly (*P*<0.05) lowest GP and CH_4 among all silages used in this study.

There were significant (P<0.05) differences between silages in terms of the estimated parameters from the GP kinetics models. The intercept value (a) for different treatments representing GP from soluble fractions ranged from -12.8 to 7.1, and the silages with 80% and 100% SS has significantly (*P*<0.001) higher instant GP from rapidly soluble fraction than other silages. The GP from the insoluble fraction (b) had a significant (*P*<0.05) linear increase, whereas, the rates of GP for the insoluble fraction (c) had a significant (*P*<0.05) linear decrease as the proportion of SS increased in the mixture silages,

4. Discussion

4.1 Chemical compositions of raw materials and SS-AF silage mixtures

The content of DM and CP in the silage mixtures is a reflection of the proportions of the original forages included in each mixture. Alfalfa is a legume and it generally contains higher level of CP than sorghum (Table 1), because of nitrogen fixation from atmosphere (Ozturk et al., 2006；Amer et al., 2012). Likewise, many authors showed that the CP content increased in maize-legume silage mixture when the proportion of legume increased (Titterton and Maasdorp, 1997; Contreras-Govea et al., 2009).

The high levels of residual WSC in the silage mixtures with more SS may be caused by the high brix and WSC in the initial SS material (Table 1), which had positive correlation with the residual WSC (Yang et al., 2006). The residual WSC was similar in 0% and 20% SS silages; this may be because the 20% SS silage provides adequate, but not excessive WSC for fermentation during ensilage. On the other hand, the increased residual WSC observed from 40% SS silage to 100% SS silage, despite the decreasing DM content, indicates that these forage mixtures supplied at least enough WSC for an effective fermentation. The content of starch in silage mixtures from this study (9 to 80 g/kg DM) is within the wide range observed from other reports. Though the forage were harvested at similar stages (milk stage for SS and early bloom stage for AF) as in the current study, Amer et al. (2012) showed lower (51 g/kg DM and 5 g/kg DM) starch content in SS silage and AF silage than in this study. This may be related to the starch content of the specific crop prior to ensilage (Table 1), which can be influenced by type of forages, culture system employed, method for ensilage, and ensilage material. For example, Colombini et al. (2012) reported a starch content of 34 g/kg DM in forage sorghum silage and 208 g/kg DM in grain sorghum silage. This is in agreement with results showed by Sang et al. (2008), who suggested that starch is a main chemical component in sorghum grain (~700 g/kg DM).

The fiber content of these silages was in agreement with those reported by other researchers (Anil et al., 2000; Qu et al., 2013). The higher fiber fractions (i.e., aNDFom, ADFom and ADL) in the SS and 100% SS silage compared with the AF and 0% SS silage may be because SS is a C_4 plant and the photosynthetic cells are arranged in Kranz structures and often contain girder structures, which collectively increases fiber content. Similar anatomical features are lacking in AF (Wilson, 1994). The higher fiber fractions (Table 1) may be necessary for SS to grow tall and to produce more biomass. The lower content of fiber in AF silage was also exaggerated by harvesting at the early-bloom stage. The quadratic effects of SS inclusion on aNDFom and ADFom indicate that up to 60% of SS can be included in the silage mixtures without increasing major fiber fractions in the silage mixtures.

The multiple phenolic hydroxyl groups in TP and CT lead to the formation of complexes with proteins, metal ions and other macromolecules like polysaccharides. These effects lead to the protection of forage proteins from degradation by inhibiting plant and microbial enzymes, resulting in better quality silages with lower NH_3 levels (Makkar, 2003). SP is a steroid or triterpene glycoside compound found in different plants. It is the main anti-nutritional components in AF plant, and their unfavourable effects on ruminant performance (such as bloat caused by production of slime from AF saponins) can restrict the optimum use of AF as an animal feed (Sen et al., 1998).

4.2 Fermentation characteristics of SS-AF silage mixtures

The fermentation characteristics indicate that adding SS in this study improved overall silage quality, with a lower pH, higher lactic acid and lower NH_3 concentration (Muck, 1988; Heron et al., 1989). These effects can be explained by the higher concentration of WSC and starch in the mixtures with a higher proportion of SS. Mono- or disaccharides that are broken down from starch can also be used as readily fermentable carbohydrate, which help to reduce pH and increase lactic acid production during the ensiling process (McDonald et al. 2002). On the other hand, the lower WSC content in silage is related to higher buffering capacity (Fisher and Burns,

1987) and extensive proteolysis during ensiling (Heron et al., 1989) may be attributed to the higher pH and NH_3 concentration with higher proportions of AF in the silage mixtures. Some research work showed a higher NH_3 concentration in maize-legume or sorghum-soybean mixtures than the maize- or sorghum- only silages (Titterton and Maasdorp, 1997; Contreras-Govea et al., 2009; Lima et al., 2010) and lower pH in Bermuda grass silages prepared from crops with higher WSC concentrations (Adesogan et al., 2004).

The higher content of acetic and propionic acids in AF silage than SS silage indicate that the legume forage was not well fermented. This was probably due to the comparatively low WSC and starch concentration in AF. Despite the lower pH was observed in silages containing 80 and 100% SS, little change was found in lactic, acetic and propionic acids. This indicates no benefit in organic acid production was obtained with more than 60% of SS in the silage mixtures. A similar change of organic acids in silage mixtures containing maize and legume have been observed (Sun et al., 2009; Zhu et al., 2011).

4.3 Ash and minerals of SS-AF silage mixtures

The higher contents of ash and the minerals such as K, Ca, P, Mg, Na, Fe and Zn in the AF silage than the SS silage were likely due to the differences that existed between SS and AF in their ability to absorb and accumulate different minerals during growing. Variation in ash and mineral concentration among crops are dependent on plant type and environmental factors (Wu et al., 2007), as well as physiological and morphological differences among plants (Hoenig et al., 1998). Interestingly, Kume (2001) found that CP in AF had a positive correlation with Ca, P, Mg and K.

4.4 In-vitro rumen degradability and fermentation of SS-AF silage mixtures

The higher IVDMD and IVOMD of silage mixtures with lower SS content may be due to their lower fiber fractions, which are known to reduce the degradability of feed (Mustafa et al., 2000; Sebata et al., 2011; Qu et al., 2013; Calabrò et al., 2010b). Moreover, the presence of higher content of phenolic compounds and tannins in sorghum silage has been found to be related to the protection of dietary protein, structural carbohydrates and starch against degradation by ruminal microorganisms (Tabacco et al., 2006; Oliveira et al., 2007). In this study, no significant difference was observed in IVDMD and IVOMD for the silage mixtures containing 0, 20 and 40% of SS. However, they all had higher degradability than 80% SS silage. This suggests that if a high degradability needs to be achieved, less than 60% SS should be added into the SS-AF silage mixtures.

The higher pH and NH_3 concentrations following the *in-vitro* incubation of low SS containing silage mixtures were expected. The higher pH and NH_3 from 100% AF silage reflects that a greater proteolysis occurred during its *in-vitro* incubation than in 100% SS silage. This is in agreement with Dhiman and Satter (1997), who reported that the ruminal NH_3 concentration was higher in cows fed AF silage than cows fed maize silage. Decreased rumen pH and NH_3 concentration have been shown in sucrose-supplemented cows (Broderick et al., 2008) and in fructose-supplemented heifers (Golder et al., 2012).

The observed increase in VFA of ruminal liquid with more AF in silage mixtures may be related to the ruminal microbe species. For example, *Fibrobacter succinogenes* mainly produces succinate, the major precursor of propionate in the rumen, while *Ruminococcus albus* mostly produces acetate (Vinh et al., 2011). The increased concentration of acetate and propionate in silage mixtures containing high level of AF may be due to the higher CP content which leads to a more favorable fermentation environment (pH, NH_3) for growth of cellulolytic bacteria. Other researchers have showed that cellulolytic bacterial population could significantly increased by higher ruminal NH_3 (Khampa et al., 2006; Vinh et al., 2011) and the cellulolytic activity of rumen contents could be markedly inhibited by a fall of pH (Terry et al., 1969; Stewart, 1977) because of their influences on the rumen ecology. Higher ruminal NH_3 level may serve as N source to improve rumen ecology (Wanapat and Pimpa, 1999). The strong positive relationship between the number of ruminal cellulolytic bacterial species and the concentration of propionate and acetate had been observed (Vinh et al., 2011). Therefore, the increase in propionate and acetate concentration that occurred in the *in-vitro* fermentation with higher AF silage might have been a consequence of the increase in number of cellulolytic bacterial, such as *Fibrobacter succinogenes*, *Ruminococcus albus*. In addition, the higher concentration of minerals in mixed silages with more AF might have contribution to the cellolytic bacterial growth (Kang et al., 2014). Similar to the findings from the current study, Lettat et al. (2013) also reported that greater ruminal pH and concentration of acetate in the rumen fluid of cows fed diet with high level of AF silage. The present finding of lower acetate production from more SS inclusion in the silages is similar to the findings from Kaplan (2011). This was likely due to the fibre type in SS that was less fermentable than that in alfalfa.

Branched-chain VFA can be derived from the fermentation of branched-chain amino acids (Saro et al., 2014), so the higher iso-butyrate and iso-valerate concentration for AF silage in this study could be due to higher CP content and its great degradation. Hassanat et al. (2014) found the ruminal concentration of branched-chain VFA increased as cows were fed higher proportions of AF silage in the diet. Also, in agreement with our results, other researchers (Haddad, 2000;

Saro et al., 2014) have reported that the rumen total VFA were increased as proportions of AF were increased in diets.

4.5 Methane and gas production of SS-AF silage mixtures

CH_4 is an end-product of rumen carbohydrates fermentation and it has been recognized as a potent greenhouse gas (Moss et al., 1994). The higher CH_4 production from silages containing less SS may have resulted from more digestible portions and lower fiber content. Blaxter and Clapperton (1965) reported that CH_4 emission was positively correlated with the amount of digestible OM. Chaudhry and Khan (2012) proved less CH_4 production for the high fibrous substrates during *in-vitro* rumen fermentation. In addition, other researchers (Tavendale et al., 2005; Bhatta et al., 2009) confirmed that tannins could suppress methanogenesis by reducing the protozoa population, which had inhibitory effects on methanogens. Methane production is higher when protozoa are present in greater numbers in the rumen than when they are present in low numbers (Bhatta et al., 2009). Thus, the lower CH_4 production in silage mixtures with lower SS had likely contributed to the stronger anti-methanogenic activity from the presence of more CT content in SS.

Over 24 hours of incubation, a higher GP was observed from the AF silage than the SS silage, this mostly likely reflected that AF had lower aNDFom, ADFom and ADL concentrations, as the negative correlation between fiber and GP was observed by Zerbini et al. (2002) and Sebata et al. (2011). Higher structural carbohydrates content can inhibit GP by limiting microbial fermentation or enzymatic hydrolysis of forage polysaccharides (Jung and Allen, 1995; Sebata et al., 2011). Sebata et al. (2011) also observed that GP was positively correlated with IVDMD and negatively correlated with CT. The trend of gas production in current study was opposite to the report from Kaplan (2011). It is likely that the AF used in this study was higher in CP content that resulted in more NH_3, which contributed to the total gas production. On the other hand, AF was low in fibre which might have caused a higher CH_4 production compared with SS. It is important to note that the GP were not different among 0%, 20%, and 40% SS silage mixtures at all times measured in this study. The shift from higher to lower GP was observed between 40% and 60% SS silage mixtures at the end of 24 hours incubation.

The higher ($P<0.001$) instant GP from rapidly soluble fraction (a) in 80% and 100% SS might reflect the more soluble fraction in SS, such as WSC. However, the negative “a” values, which are difficult to interpret in biological terms, might be due to no gas production recordings between 10 to 20 hours of incubations or possible delays in the onset of fermentation due to slow microbial colonization (Kang and Wanapat, 2013). The greater GP rate constants (c) from the insoluble but slowly degradable fraction could be a subsequence of the greater availability to the microorganisms of fermentable nutrients in the silages with more AF. The greater insoluble fractions (b) in the silages with 80% and 100%SS may be related to their higher contents of more slowly fermented fibres, such as NDFom and ADFom, which could produce more GP with longer incubation times.

5. Conclusions

Ensiling AF alone is not practical due to its high buffer capacity, pH and low WSC concentration, which make it unsuitable for producing high-quality silage. On the other hand, ensiling SS alone results in low IVDMD and IVOMD, and it indicates that the overall quality of SS-AF silage mixtures were better than would be predicted on the basis of proportional combinations of the silages prepared from SS or AF alone. Our results have demonstrated the interesting effect of mixing SS and AF for silage making on nutritive value and fermentation characteristics; it indicates that the overall quality of SS-AF silage mixtures was better than the silages prepared from SS or AF alone. The silage mixtures with SS to AF ratios of 20:80 and 40:60 have the potential to be used for ruminant production. However, additional research is needed to study the effect of feeding such silage mixtures to ruminants on their voluntary feed intake and production performance.

Acknowledgments

The financial support from the National Natural Science Foundation of China (No. 31260565; 31160472) is gratefully acknowledged. Thanks also go to technicians of Newcastle University (U.K.) and Lincoln University (New Zealand) for analyzing the samples.

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Table 1

Chemical composition (g/kg DM) of SS (sweet sorghum) and AF (alfalfa) prior to ensiling.

Crop	DM	Ash	CP	WSC	EE	Starch	aNDFom	ADFom	ADL
AF	385.93	95.83	227.05	81.63	28.14	14.95	215.82	207.24	38.58
SS	282.06	67.10	72.47	186.69	17.76	93.35	481.37	287.59	57.45
SME	23.269	6.433	34.671	23.711	5.84	17.639	59.67	18.474	4.762
P-value	<0.001	<0.001	<0.001	<0.001	0.001	0.001	0.001	<0.001	0.019

DM, dry matter; CP, crude protein; WSC, water-soluble carbohydrate; EE, ether extract; OM, organic matter; aNDFom, neutral detergent fibre in OM; ADFom, acid detergent fibre in OM; ADL, acid detergent lignin.

Table 2

Chemical composition (g/kg DM) of SS-AF (sweet sorghum-alfalfa) silage mixtures*.

Items	0%SS	20%SS	40%SS	60%SS	80%SS	100%SS	SEM	linear	quadratic
DM	393.06^{a}	386.83^{a}	354.70^{b}	333.9^{c}	305.90^{d}	286.67^{e}	9.527	<0.001	0.036
Ash	115.66^{a}	108.29^{b}	96.67^{c}	85.51^{d}	78.79^{e}	72.75^{f}	3.748	<0.001	<0.001
CP	222.77^{a}	191.35^{b}	149.79^{c}	125.20^{d}	84.88^{e}	62.32^{f}	13.628	<0.001	0.048
WSC	18.34^{e}	17.66^{e}	42.35^{d}	46.62^{c}	55.63^{b}	80.92^{a}	5.309	<0.001	<0.001
Starch	9.19^{f}	17.56^{e}	27.49^{d}	34.06^{c}	48.83^{b}	79.69^{a}	5.617	<0.001	<0.001
EE	33.25^{a}	29.74^{b}	27.87^{b}	24.73^{c}	21.26^{d}	20.34^{d}	1.138	<0.001	0.341
aNDFom	228.33^{f}	275.44^{e}	320.92^{d}	337.80^{c}	445.57^{b}	504.08^{a}	23.101	<0.001	<0.001
ADFom	211.58^{c}	221.46^{c}	226.57^{c}	221.25^{c}	273.10^{b}	305.44^{a}	8.475	<0.001	<0.001
ADL	39.94^{d}	42.56^{cd}	47.25^{bc}	46.03^{bc}	49.14^{b}	55.72^{a}	1.264	<0.001	0.175
SP	91.29^{a}	88.43^{a}	89.85^{a}	90.12^{a}	65.73^{ab}	54.84^{b}	3.994	0.001	0.019
TP	10.62^{c}	14.64^{bc}	20.39^{a}	18.11^{ab}	21.10^{a}	20.47^{a}	0.971	<0.001	0.002
CT	11.34^{b}	12.01^{ab}	12.38^{ab}	12.72^{ab}	14.75^{ab}	16.06^{a}	0.519	0.034	0.286

* Values within rows with different superscripts ($^{a, b, c, d, e}$ and ^{f}) are significantly different ($P<0.05$). DM, dry matter; CP, crude protein; WSC, water-soluble carbohydrate; EE, ether extract; OM, organic matter; aNDFom, neutral detergent fibre in OM; ADFom, acid detergent fibre in OM; ADL, acid detergent lignin; SP, saponins; TP, total phenolics; CT, condensed tannins.

Table 3

Fermentation characteristics (g/kg DM) of SS-AF (sweet sorghum-alfalfa) silage mixtures*.

Items	0%SS	20%SS	40%SS	60%SS	80%SS	100%SS	SEM	linear	quadratic
pH	5.03^{a}	4.92^{b}	4.75^{c}	4.62^{d}	4.51^{e}	4.16^{f}	0.069	<0.001	<0.001
NH_3	108.49^{a}	78.17^{b}	62.73^{c}	50.49^{d}	24.89^{e}	7.66^{f}	0.952	<0.001	0.109
Lactic acid	58.83^{c}	66.65^{c}	59.95^{c}	92.81^{b}	132.06^{a}	137.27^{a}	7.949	<0.001	<0.001
Acetic acid	65.57^{a}	68.59^{a}	67.10^{a}	66.86^{a}	63.26^{ab}	57.06^{b}	1.027	<0.001	0.001
Propionic acid	0.65^{a}	0.63^{ab}	0.56^{bc}	0.49^{c}	0.57^{bc}	0.52^{c}	0.015	<0.001	0.013

* Values within rows with different superscripts (a, b, c, d, e and f) are significantly different ($P<0.05$).

Table 4

Mineral profile (mg/kg DM) of SS-AF (sweet sorghum-alfalfa) silage mixtures*.

Items	0%SS	20%SS	40%SS	60%SS	80%SS	100%SS	SEM	linear	quadratic
K	25908.21^{a}	22412.23^{b}	16066.18^{c}	16688.41^{c}	14155.02^{cd}	12106.15^{d}	1173.507	<0.001	0.001
Ca	14340.46^{a}	12807.43^{b}	11824.76^{c}	6590.70^{d}	5324.94^{e}	3572.38^{f}	989.563	<0.001	0.051
P	2967.30^{a}	2598.49^{b}	1910.01^{c}	1831.19^{cd}	1672.83^{de}	1491.05^{e}	128.316	<0.001	<0.001
Mg	3940.92^{a}	3945.68^{a}	4064.72^{a}	3194.58^{b}	3100.73^{bc}	2969.69^{c}	111.118	<0.001	0.002
Na	1679.69^{b}	2015.40^{a}	1782.78^{ab}	903.41c	736.41^{cd}	527.59^{d}	139.571	<0.001	<0.001
Fe	717.12^{a}	696.82^{b}	622.01^{c}	505.18^{d}	444.17^{e}	423.78^{f}	28.330	<0.001	0.004
Zn	36.61^{a}	35.74^{a}	25.33^{b}	14.20^{c}	13.97^{cd}	10.54^{d}	2.569	<0.001	0.001
Mn	31.84	30.46	30.59	29.94	31.94	30.48	0.291	0.602	0.265
Cu	10.13	9.75	9.33	8.71	8.38	8.49	0.233	0.141	0.552
Mo	1.48	0.91	0.98	0.91	0.59	0.53	0.101	0.060	0.597
Co	0.25	0.25	0.23	0.22	0.19	0.19	0.008	0.081	0.874

* Values within rows with different superscripts (a, b, c, d, e and f) are significantly different ($P<0.05$).

Table 5

In-vitro degradability (g/kg DM), ammonia (g/kg DM), pH, total volatile fatty acids (m*M*) and volatile fatty acids (mol/100mol) after 24 h incubation of SS-AF (sweet sorghum-alfalfa) silage mixtures*.

Items	0%SS	20%SS	40%SS	60%SS	80%SS	100%SS	SEM	linear	quadratic
pH	6.81^{a}	6.81^{a}	6.80^{a}	6.76^{ab}	6.74^{b}	6.73^{b}	0.009	0.009	0.596
NH_3	98.46^{a}	89.31^{a}	59.65^{b}	43.79^{c}	38.90^{c}	18.39^{d}	5.972	<0.001	0.152
IVDMD	666.56^{a}	665.44^{a}	603.75^{ab}	520.69^{bc}	494.25^{c}	457.89^{c}	18.559	<0.001	0.850
IVOMD	719.91^{a}	749.03^{a}	676.10^{ab}	580.23^{bc}	552.23^{c}	498.01^{c}	21.346	<0.001	0.341
tVFA	49.79^{a}	46.89^{ab}	45.79^{ab}	45.24^{ab}	44.82^{ab}	42.43^{b}	0.793	0.003	0.840
Acetate	66.48^{a}	66.75^{a}	66.17^{ab}	66.09^{ab}	66.04^{ab}	64.58^{b}	0.853	0.014	0.925
Propionate	17.68^{a}	17.69^{a}	17.18^{ab}	16.87^{ab}	16.85^{ab}	16.50^{b}	0.259	0.039	0.420
Butyrate	9.38	9.94	10.57	11.18	11.89	12.01	0.417	0.410	0.885
iso-Butyrate	1.83^{a}	1.73^{a}	1.51^{ab}	1.48^{ab}	1.45^{ab}	1.51^{b}	0.053	0.008	0.219
Valerate	3.90^{a}	3.48^{ab}	2.99^{b}	2.76^{b}	2.79^{b}	2.90^{b}	0.119	0.007	0.148
iso-Valerate	3.00^{a}	2.77^{ab}	2.29^{b}	2.21^{b}	2.23^{b}	2.33^{b}	0.092	0.007	0.127

DM, dry matter; IVDMD, *in-vitro* DM degradability; OM, organic matter; IVOMD, *in-vitro* OM degradability; tVFA, total volatile fatty acids.

* Values within rows with different superscripts ($^{a, b, c}$ and ^{d}) are significantly different ($P<0.05$).

Table 6

In-vitro gas production, estimated parameters of gas production and methane production (mL/g DM) of SS-AF (sweet sorghum-alfalfa) silage mixtures over 24 hours incubation*.

Items	0%SS	20%SS	40%SS	60%SS	80%SS	100%SS	SEM	linear	quadratic
CH_4	25.7^{a}	24.3^{a}	23.3^{a}	24.0^{a}	20.5^{b}	21.1^{b}	0.44	<0.001	0.949
2h	23.13^{ab}	26.87^{a}	24.37^{ab}	22.50^{ab}	20.00^{b}	19.37^{b}	0.736	0.015	0.108
4h	48.75^{a}	46.88^{a}	43.75^{ab}	40.63^{ab}	37.50^{b}	35.63^{b}	1.216	0.001	0.993
6h	69.37^{a}	68.75^{a}	62.50^{ab}	59.38^{b}	50.63^{c}	40.00^{d}	2.256	<0.001	<0.001
8h	90.00^{a}	90.00^{a}	83.75^{a}	73.75^{b}	60.00^{c}	48.75^{d}	3.123	<0.001	0.001
10h	107.50^{a}	103.75^{a}	98.13^{a}	88.13^{b}	68.75^{c}	58.75^{d}	3.848	<0.001	0.001
20h	146.87^{a}	145.63^{a}	140.63^{ab}	133.13^{b}	122.50^{c}	109.38^{d}	2.889	<0.001	<0.001
22h	148.75^{a}	149.36^{a}	148.75^{a}	136.88^{b}	131.87^{b}	116.87^{c}	2.549	<0.001	0.001
24h	151.25^{a}	154.38^{a}	153.75^{a}	141.88^{b}	130.00^{c}	121.25^{d}	2.716	<0.001	0.021
Estimated parameters [#]									
a	-12.75^{c}	-5.35b^{c}	-3.84^{b}	-1.48^{b}	5.92^{a}	7.09^{a}	1.6188	<0.001	0.708
b	179.12^{b}	179.69^{b}	186.23^{ab}	176.29^{b}	231.46^{a}	233.42^{a}	8.928	0.0003	0.062
c	0.107^{a}	0.092^{a}	0.077^{ab}	0.070^{ab}	0.034^{b}	0.026^{b}	0.0063	<0.001	0.298

* Values within rows with different superscripts ($^{a, b, c}$ and ^{d}) are significantly different (*P<0.05*).

[#] a= instant gas production from rapidly soluble fraction (mL/g DM), *b* = slow gas production from insoluble fraction (mL/g DM), *c* = the rate of gas production from insoluble fraction (mL/h).