



# 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-h 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.

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**Abbreviations:** SS, sweet sorghum; AF, alfalfa; DM, dry matter; OM, organic matter; CP, crude protein; aNDFom, neutral detergent fiber in OM; ADFom, acid detergent fiber 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 carbohydrates; GP, gas production; CH<sub>4</sub>, methane; NH<sub>3</sub>, ammonia; SP, saponins; TP, total phenolics; CT, condensed tannins.

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## 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 legumes, can be mixed with low CP forages before or after ensiling. However, making silage from only legumes 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 a base 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 plants 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  $-20^{\circ}\text{C}$  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 a pH meter (pH209, Hanna Instruments., Edge, USA). Two 15 mL subsamples of the extract were centrifuged at 2500 rpm for 10 min at  $4^{\circ}\text{C}$  (MSE Mistral 3000, Sanyo Gallenkamp, Leicestershire, UK), and then acid extraction (Chaudhry and Khan, 2012) was performed on supernatant before ammonia ( $\text{NH}_3$ ) and organic acids analysis. The concentration of  $\text{NH}_3$  was analysed 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  $65^{\circ}\text{C}$  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  $\alpha$ -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 et al. (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). Water-soluble carbohydrates 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.

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