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Using mixed silages of sweet sorghum and alfalfa in total mixed rations to improve growth performance, nutrient digestibility, carcass traits and meat quality of sheep

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ABSTRACT

Combining sweet sorghum (**SS**) with alfalfa for ensiling has the potential to make a high-quality silage with relatively a better nutrient balance. However, the existing data are insufficient about how changes in ratios of SS to alfalfa in different silage mixtures affect animal performance and meat quality. Therefore, the objective of this study was to determine the effect of feeding total mixed rations containing mixed silages of SS and alfalfa on growth performance, nutrient digestibility, carcass traits and meat quality of Karakul sheep. Five total mixed rations were formulated with different SS proportions at 100%, 80%, 60%, 40%, and 20% on a fresh weight basis. Thirty 4-month old male Karakul sheep with 25.5 ± 1.4 kg BW were randomly allocated into five treatment groups, each with six lambs for this experiment. The results indicated that the Karakul sheep consuming total mixed rations containing SS-Alfalfa (**SS-AF**) silage mixtures with a lower proportion of SS tended to increase nutrient digestibility, growth performance, carcass traits, and meat quality. Feeding diets with SS at 40% and 20% inclusion rate showed a significant linear increase in the apparent digestibility of DM, CP and NDF, as well as subcutaneous fat thickness and water holding capacity ($P < 0.5$). Moreover, reduction in SS inclusion rate in these diets caused significant ($P < 0.05$) linear and quadratic increases in DM intake, final BW, average daily gain, carcass weight, and the amino acid contents of meat ($P < 0.05$). Conversely, feeding diet with lower SS inclusion rate led to decrease in feed conversion ratio and shear force significantly ($P < 0.05$) for these sheep. It appears that 40% inclusion of SS was the optimal rate in making the SS-AF silage mixture for lambs to achieve a superior production performance and high-quality meat products in Karakul sheep. However, further research is needed to investigate the effect of feeding SS-AF silage mixtures alongside contrasting ingredients on rumen function, ruminal microorganisms and digestive enzyme activity of sheep and other ruminant animals.

Implications

Combining sweet sorghum with alfalfa for ensiling has the potential to make a high-quality silage. However, how changes in ratios of sweet sorghum to alfalfa in silage mixtures affect animal performance and meat quality is still unclear. Our study results showed that 40% inclusion of sweet sorghum in sweet sorghum-alfalfa silage mixture was the optimal rate to support a high production performance and high meat quality in Karakul sheep. This study provides useful information and a scientific reference for further application of sweet sorghum-alfalfa silage mixtures in total mixed rations to reduce nutrient wastage and promote sustainable ruminant production.

Introduction

The shortage of high-quality roughage resources is the main factor restricting the development of sheep industry in many developing countries. Sweet sorghum (*Sorghum bicolor*, **SS**) is a

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promising forage growing in the arid, semi-arid and high salinity areas because of its high growth rate, biomass yield and water-soluble carbohydrate (**WSC**) content (Wu et al., 2010; Qu et al., 2014). The SS is also known to be stress resistant, drought tolerant and highly water-use efficient (Rooney et al., 2007). The SS has high fermentability, nutrient digestibility and palatability (Calabrò et al., 2010; Amer et al., 2012; Campanili et al., 2017). However, the CP content (62–100 g/kg DM) in SS silage (Colombini et al., 2012; Zhang et al., 2015) is insufficient to maximize growth efficiency in most ruminant production systems (National Research Council (NRC), 2007).

Alfalfa (*Medicago sativa*) is commonly used as a cultivated protein-rich legume. To preserve nutrients and improve palatability, making alfalfa silage for its feeding to ruminants has been an increasingly popular practice worldwide. However, making alfalfa silage without using additives or mixing with other forages is a challenging task, because of its low content of WSC, high buffering capacity (Plaizier, 2004) and extensive proteolysis during ensilation (McDonald et al., 1991).

In theory, through utilizing complementary advantages of SS and alfalfa, it is possible to make SS-Alfalfa (**SS-AF**) silage mixtures to provide a suitable diet or basal diet with balanced energy and protein for ruminant production. A previous *in vitro* study (Zhang et al., 2015) had shown that the fermentation and nutritional quality of SS-AF silage mixtures could be optimal when the ratios were 20:80 and 40:60. However, little is known about the effect of feeding different ratios of SS-AF silage mixtures on animal performance. Therefore, this comprehensive study aimed to investigate the effects of feeding different proportions of SS-AF silage mixtures on sheep nutrient digestibility, growth performance, carcass traits and meat quality. Moreover, this study allows us to determine the optimal proportion of SS for its use to prepare sustainable diets for growing lamb production.

Material and methods

Silage mixture and experimental diets

The variety of Cowley SS with 22.5% Brix value and *Hetian Bigleaf* alfalfa were sown at the Agricultural Research Station of Tarim University, XinJiang, China (longitude 81°31́E, latitude 40°56́N). The whole plant of SS at the milky stage and alfalfa at the bloom stage (10% flowering rate) was harvested and chopped into 2.5 cm particle size by a multi-functional chopper (9DF53, Yanbei Animal Husbandry Machinery Group Co. Ltd., Beijing, China). A silage wrapping machine (JD5552, Qufu Tianliang Trading Co. Ltd, Shandong, China) was used for making five types of SS-AF silage mixtures with different proportions of SS: alfalfa (20%, 40%, 60%, 80% and 100% SS based on fresh weight). All the wrapped silage was stored at room temperature for 60 days until feeding.

The experimental diets were designed according to the nutritional requirements of a lamb with 25 kg of BW (NY/T 816-2004) and comprised 40% SS-AF silage mixture. The nutrient levels of the diets as total mixed ration, were tested. The contents of DM, CP, and ether extract (**EE**) were determined according to Association of Official Analytical Chemists (AOAC, 2004) procedures, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991). The N content was determined using an Elementar Vario Macro Cube (Elementar, Hanau, Germany), and the CP content was calculated by multiplying the N with 6.25. The concentrations of calcium (Ca) were determined by atomic absorption spectrophotometry and phosphorus (P) was determined by colorimetry (Pollman, 1991). Metabolizable energy (**ME**) was calculated according to the Small Ruminant Nutrition System (Tedeschi et al., 2010). The composition and nutrient levels of the diets are shown in table 1.

Animals

The experiment was carried out at the Animal Research Station of Tarim University, XinJiang, China. All procedures used in this study were performed according to the Guidelines for the Care and Use of Animals for Research in China (GB 14925-2001).

Thirty 4-month old male Karakul sheep, with an initial mean BW of 25.5 ± 1.4 kg, received 2.5 mL/animal of levamisole chloride for prophylactic management against internal parasites, before they were randomly allocated into five dietary treatment groups in a completely randomized design. All animals were housed in individual stalls (2.4 m × 1.2 m), which were equipped with feeders and water buckets. Sheep had *ad libitum* access to clean water and respective experimental diets throughout the experiment. The experimental diets were formulated with a small total mixed ration mixer (RH-IBJ-400, Qufu Runhua Machinery Manufacturing Co. Ltd, Shandong, China).

The experiment lasted for 80 days, with 10 days of adaptation to feeding and housing routines, 60 days for growth performance and the last 10 days for measurement of nutrient digestibility as described below.

Growth performance and apparent nutrient digestibility

The experimental diets were individually offered *ad libitum* to the animals twice a day at 09:00 and 19:00, and the offered amount was adjusted to achieve approximately 10% refusal. Feed offered and refused were recorded twice a day to achieve the feed intake potential of each animal and calculate DM intake (**DMI**). The lambs were weighed on growth measurement days of 0, 20, 40, and 60 before their morning feed to calculate average daily gain (**ADG**) and feed conversion ratio (**FCR**).

A digestibility trial was conducted from experimental d 71 to d 80 of the experiment period with 5 days of adaptation and a subsequent 5 days of collection of feces. All the lambs were housed in individual metabolism cages (1.2 m × 1.5 m) with a plastic-screen sitting underneath the cage for fecal collection. The fecal output was collected and quantified from each sheep before offering each meal daily. Approximately 15% of the diets and fecal samples from each animal were collected daily from experimental d 76 to d 80 of the experiment period, and 10 mL of 10% (vol/vol) sulfuric acid was added to the fresh feces from each lamb to prevent ammonia nitrogen volatilization. All the samples were stored immediately at −20 °C until analyzed for proximate composition. Apparent nutrient digestibility was determined as: [(nutrient consumed - nutrient in feces)/nutrient consumed] × 100%.

After drying in an oven at 65 °C for 48 h to determine DM content, samples of diet and feces were ground through a 1 mm sieve using a mill (Christy and Norris Co. Ltd., Suffolk, UK) prior to chemical analysis. The CP, NDF, ADF, and EE contents were determined using the same methods as described above, and acid detergent lignin (ADL) was determined according to Van Soest et al. (1991).

Animal slaughter and carcass traits

At the end of the experiment, the experimental sheep were fasted for 24 h with free access to water and transported to a commercial abattoir (Xinjiang Tianshan Xuelong Animal Husbandry Technology Co., Ltd.) located about 10 km away from the experimental site. The sheep were stunned and slaughtered humanely at the abattoir according to standard commercial procedures (NY 467-2001, The Ministry of Agriculture of the People's Republic of China).

Table 1
Ingredients and nutrient compositions of sweet sorghum-alfalfa (SS-AF) silage-based diets of Karakul sheep.

Item	SS percentage in the silage mixture				
	100%SS	80%SS	60%SS	40%SS	20%SS
Ingredients, %					
Corn	13	13	13	13	13
Wheat bran	10	10	10	10	10
Soybean meal	12	12	12	12	12
Premix[1]	5	5	5	5	5
Cottonseed hull	10	10	10	10	10
Rice straw	10	10	10	10	10
Silage mixture	40	40	40	40	40
Total	100	100	100	100	100
Nutrients, %					
DM	66.13	65.91	66.03	66.86	66.14
CP	10.50	10.80	11.18	12.39	12.75
EE	2.20	2.24	2.38	2.50	2.58
NDF	55.51	54.18	52.81	50.70	50.38
ADF	27.89	25.73	24.73	24.03	24.01
Ca	1.22	1.13	1.31	1.10	1.24
P	0.84	0.81	0.84	0.86	0.80
ME, MJ/kg	9.06	9.22	9.54	9.71	9.93

SS = sweet sorghum; EE = ether extract; ME = metabolizable energy.
[1] The premix provided the following per kg of diets: Vitamin A (IU) = 9 750, Vitamin D_3 (IU) = 2 450, Vitamin E (IU) = 19.5, nicotinic acid (mg) = 11.5, Fe (mg) = 66, Zn (mg) = 70, Mn (mg) = 38, S (mg) = 0.4, Se (mg) = 0.3, Ca (g) = 7.5, P (g) $\geq$ 0.75, NaCl (g) = 9.

The weight of hot carcass, liver, heart, spleen, kidney, and kidney fat was recorded after slaughtering according to anatomical site (Akers and Denbow, 2013); and the records were used to evaluate the carcass traits. The organ weight percentage relative to empty BW was calculated as follows: percentage relative to empty BW = (the organ weight/empty BW) × 100%. Furthermore, hot carcass weight was used to calculate the hot carcass yield: carcass yield = (hot carcass weight/live weight) × 100%.

A planimeter (KP-21C, Koizumi Co. Ltd., Tsuchiura, Japan) was used to determine the cross-sectional area of the Longissimus dorsi muscle (**LDM**) at the 12th and 13th rib interface on sulfur paper. Subcutaneous fat thickness was measured by a Vernier caliper (Booher-303215, Kunshan Kaipai Hardware and Electrical Co. Ltd., Jiangsu, China) at this same site of each selected animal.

Meat quality and chemical analysis

Approximately 200 g samples of LDM between the 9th and 11th ribs were collected from the right side of each carcass and used to evaluate meat quality and measure chemical composition. Meat quality was examined by determining the muscle pH, meat color, water holding capacity (**WHC**), cooking loss, and shear force. Muscle pH values at 0 h (pH_0) and 24 h (pH_{24}) after slaughter were tested using a pH meter (CD700, Russell pH Limited, Germany) whereas color, cooking loss, and shear force were performed according to Honikel (1998).

Meat color CIE lightness (**L***), redness (**a***), and yellowness (**b***) values were measured from three different places of the LDM by using a chromameter (Konica Minolta, CR-400, Japan). The LDM samples were cooked in a temperature-controlled water bath in plastic bags to an internal temperature of 75 °C using a digital thermometer to measure the internal temperature of the sample. The samples were weighed before and after cooking. Cooking loss was calculated from the difference in the weight of raw and cooked samples and was expressed as a percentage of initial weight. The cooked samples were stored at 4 °C for 12 h after determining the cooking loss and cut into 1 cm^3 cube to conduct a texture profile analysis. The shear force of the cylinder was measured using a texture analyzer (Stable Micro System, Surrey, United Kingdom) set at a speed of 200 mm/min, measured in kg/cm^2.

The WHC was tested by the method described by Franco et al. (2009). In brief, 10 g of meat sample was wrapped with double gauze and padded 18 layers of filter paper on the top and bottom of the meat samples, then placed under 35 kg of pressure for 5 min, and the mass was immediately weighed. The WHC was calculated as follows: WHC = [(total muscle weight − meat loss)/total muscle moisture] × 100%. The chemical composition including DM, CP, and EE was determined using the same methods as described above, and ash was determined using a muffle furnace at 550 °C for 5 h.

Amino acid analysis

Amino acid composition of the LDM was determined using a high-performance liquid chromatography system (Waters 2695 Technologies, USA), equipped with a quaternary pump delivery system, robotic autosampler, thermostat column compartment and a diode array detector. Approximately 100 mg of minced muscle samples were hydrolyzed with 20 mL of HCl (6 mol/L) at 110 °C for 22 h in sealed evacuated tubes.

The samples were submitted to automatic precolumn derivatization. After derivatization, 10 μL of each sample was injected into the high-performance liquid chromatography to determine amino acid composition. Mobile phase A was 40 mM NaH_2PO_4, adjusted to pH 7.8 with NaOH, while mobile phase B contained 45 acetonitrile, 45 methanol, and 10% deionized water. The chromatographic column temperature was set at 37 °C with a flow rate of 1 mL/min. The identity and quantity of the amino acids were assessed by comparison with the retention times and peak areas of standard amino acids.

Statistical analysis

The SPSS statistical package (version 17.0; SPSS Inc., Chicago, USA) was used for statistical analysis of all the data. One-way ANOVA was used to examine the linear and quadratic effects of silage types on growth performance, nutrient digestibility, carcass traits, meat quality, and amino acid composition. The Tukey's post-hoc test was used for multiple comparisons of means. In this study, all the data were presented as the mean values and SEM. Treatment differences were considered to be significant when $P < 0.05$.

Results

Growth performance

The effects of SS-AF silage mixtures on the BW, ADG, DMI, and FCR of Karakul sheep are shown in Table 2. The BW, ADG, and DMI of the animals on d 60 showed a significant (P < 0.05) linear increase as the proportion of SS declined in the SS-AF silage mixtures, and the values were significantly (P < 0.05) higher with a proportion of SS at 20% and 40% in the SS-AF silage mixtures than those with SS at 100% proportion. The highest BW, ADG, and DMI were obtained with a diet including the 40% SS silage mixture which was 1.14, 1.66, and 1.19 times higher (P < 0.05) than the 100% SS silage-based diet, respectively (Table 2).

There was a tendency for a decrease in FCR as the proportion of SS decreased from 100% to 20% in the SS-AF silage-based diet on d 20 and d 40. The FCR responded with a significant (P < 0.01) linear decrease for sheep fed with diets containing 40% and 20% SS in the SS-AF silage-based diets on d 60. During the whole experiment (0–60 d), diets including mixed silage with 40% SS promoted the highest final ADG and DMI, and the lowest value of FCR (P < 0.05).

Apparent nutrient digestibility

The effects of SS-AF silage mixtures on apparent nutrient digestibility are shown in Table 3. The apparent digestibility of DM and CP with diets including silage mixtures with SS at 40% and 20% inclusion rate were significantly higher (P < 0.05) than those with SS at 100% and 80% inclusion rate, and the NDF digestibility of diets with alfalfa in the mixed silages responded in significant (P < 0.05) linear and quadratic increases compared with the diet containing 100% SS silage. The apparent digestibility of ADF, ADL and EE were not affected by the change in the proportion of SS in silage mixtures (P > 0.05).

Carcass traits

The effects of SS-AF silage-based diets on carcass traits are shown in Table 4. The carcass weight and subcutaneous fat thickness of Karakul sheep fed with diets including silage mixtures with SS at 20%, 40%, and 60% supported a significant response (P < 0.01) which was higher than those with SS at 100% inclusion rate. The 40% inclusion rate of SS promoted the highest carcass weight and subcutaneous fat thickness. There was a tendency for an increase for the LDM area as the proportion of SS decreased from 100% to 20% in SS-AF silage mixtures in the diet, although it did not reach significance (P = 0.09). Nevertheless, changing the proportion of SS in silage mixtures had no significant effect on carcass yield and relative weight of liver, heart, spleen, kidney, and kidney fat (P > 0.05).

Meat quality and chemical composition

The effects of SS-AF silage-based diets on meat quality and chemical composition are shown in Table 5. Dietary treatment had a linear effect on the contents of CP and EE, as well as the values for lightness, redness, shear force, and WHC in meat as the proportion of SS decreased in the SS-AF silage-based diet. Also, the proportions of SS at 40% and 20% in the SS-AF silage mixtures had significantly higher (P < 0.05) values for CP, EE, lightness and WHC, but had significantly (P < 0.01) lower shear force compared with 100% SS inclusion rate. For pH, water and ash content, yellowness, cooking loss of meat, no significant (P > 0.05) differences were observed.

Amino acid composition

The effects of SS-AF silage-based diets on the contents of seventeen amino acids of the LDM from Karakul sheep are shown in Table 6. The contents of glutamic, cysteine, threonine, lysine and total amino acids had a significant (P < 0.05) linear increase in the meat of sheep fed with silage-based diets including SS at 40% than those including SS at 80% or 100%. Conversely, the other amino acids were not affected by diets.

Discussion

Our previous research demonstrated that the overall nutrient composition of SS-AF silage mixtures was changed. As the proportion of SS decreased in the silage, the concentrations of Ash, CP and EE increased, whereas the concentrations of ADF, NDF, WSC, phenolics and tannins were decreased (Zhang et al, 2015).

Table 2
Effects of sweet sorghum (SS) proportion in sweet sorghum-alfalfa (SS-AF) silage mixture on growth performance of Karakul sheep.

	SS percentage in the silage mixture							
Item	100%SS	80%SS	60%SS	40%SS	20%SS	SEM	Linear	Quadratic
BW, kg								
0 d	26.1	26.1	25.6	25.7	26.3	0.65	0.53	0.15
20 d	28.5	29.1	29.6	30.6	29.6	0.62	0.17	0.22
40 d	31.5	33.2	34.1	34.6	33.4	0.62	0.06	0.03
60 d	33.6[c]	35.7[b]	36.9[a]	38.2[a]	37.0[a]	0.69	<0.01	0.03
ADG (average daily gain), g/animal per day								
20 d	125[c]	155[bc]	197[ab]	242[a]	163[bc]	29.26	<0.01	0.01
40 d	146	201	224	203	193	31.06	0.17	0.04
60 d	107[b]	127[b]	141[ab]	181[a]	179[a]	19.60	<0.01	0.72
0–60 d	126[c]	161[b]	187[ab]	208[a]	178[ab]	15.76	<0.01	<0.01
DMI (DM intake), g/animal per day								
20 d	816[b]	897[a]	870[a]	911[a]	880[a]	24.11	<0.01	0.02
40 d	768[c]	852[b]	881[ab]	941[a]	912[ab]	42.53	<0.01	0.10
60 d	771[b]	821[ab]	852[ab]	912[a]	881[a]	29.33	<0.01	0.05
0–60 d	775[b]	847[ab]	861[b]	924[a]	891[a]	21.12	<0.01	<0.01
FCR (feed conversion ratio)								
20 d	6.8	6.2	4.6	4.3	5.7	0.90	0.05	0.13
40 d	5.8	4.4	4.2	4.9	5.0	0.72	0.24	0.15
60 d	7.7[a]	6.8[ab]	6.2[ab]	5.5[b]	5.0[b]	0.75	0.01	0.62
0–60 d	6.4[a]	5.4[b]	4.8[b]	4.6[b]	5.1[b]	0.40	<0.01	<0.01

In the same row, mean values with different superscripts were significantly different (P < 0.05).

Table 3
Effects of sweet sorghum (SS) proportion in sweet sorghum-alfalfa (SS-AF) silage mixture on nutrient digestibility of Karakul sheep.

Item	SS percentage in the silage mixture					SEM	Linear	Quadratic
	100%SS	80%SS	60%SS	40%SS	20%SS			
DM, %	60.4[c]	62.1[b]	63.7[b]	67.5[a]	65.8[a]	0.93	0.04	0.51
CP, %	64.4[b]	65.2[b]	68.8[a]	72.3[a]	71.9[a]	0.97	0.01	0.48
NDF, %	54.6[b]	61.2[a]	61.6[a]	63.3[a]	59.8[a]	1.01	0.03	0.01
ADF, %	61.1	54.4	51.3	59.4	58.8	1.36	0.10	0.13
ADL, %	5.2	5.4	5.0	5.4	5.1	0.12	1.25	0.19
EE, %	84.9	83.6	84.1	84.3	85.0	5.48	0.68	0.94

EE = ether extract.
In the same row, mean values with different superscripts were significantly different ($P < 0.05$).

Table 4
Effects of sweet sorghum (SS) proportion in sweet sorghum-alfalfa (SS-AF) silage mixture on carcass traits and organ weights of Karakul sheep.

Item	SS percentage in the silage mixture					SEM	Linear	Quadratic
	100%SS	80%SS	60%SS	40%SS	20%SS			
Carcass traits								
Carcass weight, kg	15.9[c]	17.1[bc]	18.8[ab]	20.3[a]	19.2[ab]	0.50	<0.01	<0.01
Carcass yield, %	48.3	47.9	48.9	51.5	49.3	0.70	0.58	0.46
LDM area, cm^2	16.6	16.4	17.9	18.3	17.7	0.74	0.09	0.33
Subcutaneous fat, mm	1.51[b]	1.54[b]	1.58[a]	1.60[a]	1.59[a]	0.02	<0.01	0.05
Organ weight, g								
Liver	590	625	617	608	639	34.93	0.71	0.88
Heart	183	175	189	199	190	19.36	0.68	0.89
Spleen	40	41	41	45	39	5.77	0.99	0.50
Kidney	100	105	111	106	118	7.58	0.22	0.90
Kidney fat	276	212	218	203	209	27.98	0.09	0.10
Percentage relative to empty BW,[1] %								
Liver	1.80	1.76	1.60	1.53	1.65	0.08	0.12	0.50
Heart	0.56	0.49	0.49	0.51	0.49	0.04	0.45	0.31
Spleen	0.12	0.12	0.11	0.11	0.10	0.02	0.72	0.99
Kidney	0.31	0.30	0.30	0.27	0.30	0.01	0.36	0.12
Kidney fat	0.67	0.60	0.57	0.51	0.54	0.08	0.06	0.12

LDM = longissimus dorsi muscle.
In the same row, mean values with different superscripts were significantly different ($P < 0.05$).
[1] Percentage relative to empty BW (%) = (the organ weight/empty BW) × 100%.

Table 5
Effects of sweet sorghum (SS) proportion in sweet sorghum-alfalfa (SS-AF) silage mixture on meat quality of Karakul sheep.

Item	SS percentage in the silage mixture					SEM	Linear	Quadratic
	100%SS	80%SS	60%SS	40%SS	20%SS			
Water, %	74.1	74.2	74.4	74.5	74.9	0.36	0.24	0.56
Ash, %	1.30	1.28	1.20	1.27	1.23	0.07	0.23	0.71
CP, %	68[b]	78[a]	83[a]	84[a]	82[a]	4.46	0.03	0.05
EE, %	4.2[b]	4.2[b]	4.7[ab]	5.3[a]	5.6[a]	0.39	0.01	0.54
pH_0	6.23	6.31	6.22	6.25	6.35	0.06	0.87	0.69
pH_{24}	5.43	5.41	5.40	5.40	5.45	0.02	0.97	0.56
Lightness, L*	33[c]	34[bc]	35[b]	38[a]	36[ab]	1.00	<0.01	0.08
Redness, a*	12.4[c]	13.5[abc]	14.2[ab]	15.1[a]	13.3[bc]	0.71	0.03	0.01
Yellowness, b*	3.8	4.1	3.9	3.5	3.7	0.35	0.40	0.56
Cooking loss, %	37.5	37.4	35.9	34.7	35.5	0.82	0.83	0.77
Shear force, kg/cm	5.6[a]	5.3[ab]	5.0[bc]	4.8[cd]	4.8[cd]	0.21	<0.01	0.75
WHC, %	5.3[b]	5.2[b]	6.3[a]	6.3[a]	6.4[a]	0.33	<0.01	0.58

EE = ether extract; WHC = water holding capacity.
In the same row, mean values with different superscripts were significantly different ($P < 0.05$).

The higher nutrient digestibility with lower SS proportion (40% and 20%) in the SS-AF silage mixtures may be due to their lower fiber content that is known to increase the digestibility of feed (Mustafa et al., 2000; Sebata et al., 2011; Qu et al., 2013; Calabrò et al., 2007). Interestingly, this is in agreement with Gebregiorgis et al. (2017), who reported that the nutrient digestibility was higher in sheep fed a diet with higher CP and lower ADF and NDF contents. Conversely, the lower nutrient digestibility of SS-AF silage mixtures with a higher proportion of SS (100% and 80%) may be due to the higher phenolic and tannin contents in SS than that in alfalfa (Zhang et al, 2015). Phenolic compounds and tannins had been proven to inhibit the degradation of dietary protein, structural carbohydrates and starch by ruminal microorganisms (Tabacco et al., 2006; Oliveira et al., 2007).

The higher levels of BW, ADG and DMI obtained by Karakul sheep fed the diet with lower SS content in the SS-AF silage

Table 6
Effects of sweet sorghum (SS) proportion in sweet sorghum-alfalfa (SS–AF) silage mixture on amino acids in sheep meat.

Item	SS percentage in the silage mixture					SEM	Linear	Quadratic
	100%SS	80%SS	60%SS	40%SS	20%SS			
non-essential amino acid, % fresh matter based								
Aspartic	1.19	1.20	1.22	1.23	1.24	0.10	0.97	0.95
Serine	0.39	0.40	0.44	0.46	0.45	0.03	0.22	0.60
Glutamic	1.92[b]	1.96[b]	1.99[b]	2.31[a]	2.46[a]	0.11	<0.01	0.13
Glycine	0.68	0.68	0.71	0.72	0.68	0.05	0.93	0.54
Histidine	0.47	0.49	0.50	0.54	0.49	0.02	0.18	0.14
Arginine	1.03	1.04	1.20	1.21	1.13	0.07	0.08	0.11
Alanine	0.80	0.80	0.87	0.92	0.87	0.04	0.08	0.45
Proline	0.62	0.57	0.60	0.63	0.59	0.08	0.19	0.15
Cysteine	0.05[c]	0.05[bc]	0.06[ab]	0.06[a]	0.06[ab]	<0.01	0.01	0.14
Tyrosine	0.42	0.42	0.46	0.47	0.46	0.02	0.40	0.44
Essential amino acid, % fresh matter based								
Methionine	0.20	0.22	0.22	0.22	0.23	0.01	0.92	0.89
Threonine	0.90[b]	0.93[b]	1.02[ab]	1.08[a]	0.97[ab]	0.05	0.04	0.06
Valine	0.48	0.48	0.49	0.50	0.49	0.01	0.75	0.68
Lysine	2.10[c]	2.16[bc]	2.30[ab]	2.37[a]	2.25[ab]	0.06	0.01	0.03
Isoleucine	0.39	0.39	0.44	0.44	0.44	0.02	0.11	0.37
Leucine	0.65	0.69	0.70	0.70	0.69	0.02	0.26	0.15
Phenylalanine	0.41	0.42	0.44	0.44	0.44	0.01	0.39	0.31
Total amino acids, %	12.64[c]	12.90[c]	13.65[b]	14.28[a]	13.93[b]	0.14	<0.01	<0.01

In the same row, mean values with different superscripts were significantly different ($P < 0.05$).

mixtures were likely to be a result of feeding higher levels of dietary CP and lower fiber contents compared with those lambs receiving high SS based diets (Table 1). The higher growth performance in sheep fed lower SS based diets was a reflection of the higher nutrient digestibility of those SS-AF silage-based diets, as well as the higher DMI, as some researchers have shown that dietary protein could significantly influence the diet intake, health and growth performance of livestock (Zhang et al., 2014; Peng et al., 2018; Balasubramanian et al., 2020; Wang et al., 2020b). Interestingly, dietary CP content had a positive correlation with the BW, ADG, and DMI of ruminant animals (Hajji et al., 2016; Zhang et al., 2017).

The low FCR caused by the presence of lower SS in the SS-AF silage-based diets indicated that the quality of diets could be improved by including more alfalfa in the SS-AF silage-based diets. In this study, the highest ADG, DMI and the lowest FCR were obtained with 40% rather than the 20% SS inclusion rate, which demonstrated that better quality diets could be achieved with 40% SS in the SS-AF silage-based diets by supplying more balanced energy and protein for sheep (Santana et al., 2019; Kintl et al.,2019; Kennedy et al., 2018). The growth performance in lambs fed SS at 20% in SS-AF silage-based diet was not as high as that of SS at 40%. It may be due to the mild contamination and mildew caused by excessive CP and inadequate WSC in the SS-AF silage mixture with 20% SS inclusion. Our previous study also proved that the residual WSC in 0% SS silage and 20% SS silage was 18.34 g/kg and 17.66 g/kg respectively, which were considered inadequate for a desirable silage fermentation (Zhang et al, 2015). A forage with high protein and low WSC concentration is unsuitable to produce high-quality silage because of the high buffer capacity, high pH value, low WSC and extensive proteolysis found during ensiling (Fisher and Burns, 1987; McDonald et al., 1991). With this consideration in mind, alfalfa was excluded from its sole use to make a silage in this experiment.

Carcass traits and meat quality are crucial indices reflecting a desirable livestock production in animal husbandry (Silva et al., 2019; Luo et al., 2019) because those are closely associated with the dietary composition (Francisco et al., 2020). The higher values of CP, EE, lightness, redness, WHC in meat, carcass weight as well as subcutaneous fat thickness of Karakul sheep fed diets containing silage mixtures with SS at 20%, 40%, and 60% inclusion may have resulted from a higher concentration and reasonable balance of CP and ME in diets (Table 1). The fact that animals receiving a lower percentage of SS gained more weight and accumulated more fat in this experiment might also have influenced the meat color. Likewise, other researchers have shown that the live weight and carcass weight increased significantly with high dietary energy (Wang et al., 2020a; María et al., 2020) and high dietary protein (Wang et al., 2020b).

Compared with other percentages of SS inclusions, the lambs fed with silage mixture including 40% SS had the largest LDM area in this study, indicating that the silage made with 40% SS and 60% alfalfa had the highest feed utilization rate resulting in the higher lean meat rate. LDM area provides a good indication of meat production performance of livestock (Suzuki et al., 2005). The lower shear force representing tenderness of meat in sheep fed with diets containing silage mixtures with 20% and 40% SS (Table 5) might be due to the more subcutaneous fat thickness, which could increase the amount of intramuscular fat (Knecht and Duzinski, 2016). Increased intramuscular fat could reduce the physical strength of muscle fiber and make the muscle bundle easy to separate. As a result, the muscles become softer and easier to chew for a consumer. Intramuscular fat is useful to improve flavor which improves the consumer acceptance of products (Kouba and Bonneau, 2009). Consistent with the results of this study, previous studies have shown that lower shear force also could be obtained by a higher content of protein (Protes et al., 2018) and energy (Abouelezz et al., 2019) in the diet.

Amino acids play an important role in meat quality by providing human required nutrient and flavor characteristics (Cai et al., 2010). There is a limited literature about the effects of SS-AF mixed silage-based diets on the concentration of amino acids in meat. In the present study, the contents of glutamic, cysteine, threonine, and lysine increased significantly with the proportion of SS at 40% in the SS-AF silage mixture. This result, which is difficult to interpret in biological terms, might be due to the more protein with a modified amino acid profile in silage mixtures with less SS and more alfalfa inclusion. Wang et al. (2020b) proved that high protein diets could increase the contents of amino acids in LDM of Tibetan Sheep. On the other hand, glutamic acid has been recognized as an important flavor amino acid (Tian et al. 2020), while threonine and lysine are essential amino acids that are required to support the human body (Grabez, et al., 2020). Therefore, 20%

and 40% SS proportion in the SS-AF silage mixtures could be used in diets of sheep to improve their well-being, growth and meat quality that is beneficial to enhance flavor in meat and support human health.

Conclusion

Based on the results, lower proportions of SS in the SS-AF silage-based diets appeared to improve growth performance, nutrient digestibility, carcass traits and meat quality for Karakul sheep. The high quality of SS-AF silage mixtures with SS at 40% and 20% inclusion was determined in this animal experiment, which is consistent with the results obtained by the methods of chemical analysis, silage fermentation traits and *in vitro* digestion determined in our previous study. In summary, overall considering the results of this experiment and actuarial production, the inclusion of SS at a rate of 40% is recommended as the optimal proportion in the SS-AF silage mixtures to formulate diets for sustainable sheep production.

Ethics approval

All procedures used in this study were performed according to the Guidelines for the Care and Use of Animals for Research in China (GB 14925-2001).

Data and model availability statement

None of the data were deposited in an official repository. Upon reasonable request, the data and statistical models are available from the corresponding author.

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Declaration of interest

We declare that there is no conflict of interest in the subject of this manuscript.

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