Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Effect of corn and millet silage and their particle size on feed intake, digestibility, rumen parameters, and feed intake behavior in Kermani sheep

Leila Turkestani[1], Mohammad Mehdi Sharifi Hosseini[1], Reza Tahmasbi[1], Ali Maddahian[2], Omid Dayani[1]*

[1]*Department of Animal Sciences, Faculty of Agriculture, Shahid Bahonar University of Kerman, Iran.*
[2]*Animal Science Research Department, Gilan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran.*

*Corresponding author,
E-mail address: mmsharifi@uk.ac.ir
Tel: +989204201335

Received: 30 May 2023,
Accepted: 06 Aug 2023,
Published online: 09 Sep 2023,

ORCID
Leila Turkestani
0000-0003-2368-8760
Mohammad Mehdi Sharifi Hosseini
0000-0002-4713-6373
Reza Tahmasbi
0000-0002-5211-657X
Ali Maddahian
0000-0002-7674-4888
Omid Dayani
0000-0002-7067-8242

Abstract The purpose of this experiment was to study the effects of silage type (ST) and levels of silage particle size (SPS) on feed intake, digestibility, rumen parameters, and feed intake behavior in Kermani sheep. Corn and millet crops were cultivated in May and harvested into coarse and fine forages in mid-September. Four rams (two years old, BW 39.2±3.1 kg) were randomly assigned to four treatments in a 2×2 factorial arrangements in a Latin square design. The experimental diets were coarse corn silage diet; short corn silage diet; coarse millet silage diet and short millet silage diet. Diets were iso-nitrogenous and iso-energetic, and the ratio of forage to concentrate was 50:50. The potential of gas production was higher in corn silage (CS) than millet silage (MS) (P<0.03) and coarse SPS than short SPS (P<0.02). The dry matter (DM), organic matter (OM) and crude protein (CP) intakes were higher in short SPS diets while DM, OM and neutral detergent fiber (NDF) digestibility were higher in CS diets (76.26, 73.43 and 58.71%, respectively), while the CP digestibility was higher in short SPS diets (74.98%). The mean ruminal pH value was lower in CS diets (P<0.05). The mean ruminal ammonia nitrogen (NH_3-N) concentration was higher in CS and short SPS diets. Microbial protein (MP) synthesis was higher in short SPS diets (P<0.05). Chewing activity was higher in MS and short SPS diets (P<0.05). The results indicated the higher quality of CS, although MS can be safely fed to small ruminants such.

Keywords: chewing activity, eating activity, microbial protein, protozoa

Introduction

Silages are popular forage, and preferred to dry forages for dairy farmers due to lower nutrient loss from harvesting to storage, easier feeding, and often greater efficiency and timelines of feed mixing and handling on the farm (Grant and Ferraretto, 2018). Silage has been used as a sole forage source or partial forage source to determine the effects of physically effective neutral detergent fiber (peNDF) in numerous studies (Teimouri Yansari et al., 2004). Corn (*Zea mays*) silage is a major forage component in dairy cattle diets, principally due to its high dry matter (DM) yield, single cut harvest at optimum DM contents, high net energy for lactation (NE_l), capacity to sustain high milk yields, and good ensiling characteristics (Brunette et al., 2014).

Restriction of water resources has resulted in problems to produce good quality forage, which has led to efforts to cultivate alternative corn products in Iran. Common millet (*Panicum miliaceum*) is a suitable alternative forage for planting in areas with limited water resources (Zegada-Lizarazu and Iijima, 2005). Millet is a C4 plant that has a very high photosynthetic efficiency and DM production capacity. It is usually grown under more adverse agro-climatic conditions where other crops fail to produce economic yields (Berwal et al., 2017). The results of some studies indicated that increasing peNDF increases the chewing activity and rumen pH and decreases the risk of ruminal acidosis (Krause et al., 2002).

Numerous studies have been conducted separately on particles size of CS or MS in the diet of dairy cattle (Teimouri Yansari et al., 2004; Ferraretto and Shaver, 2012; Brunette et al., 2014; Grant and Ferraretto, 2018), with only a few studies being carried out in small ruminants (Khan et al., 2010; Sharifi et al., 2012). Therefore, the objective of this study was to examine the effects of silage type and level of silage particle size on feed intake, digestibility of nutrients, rumen parameters, and feeding behaviors in Kermani sheep.

Materials and methods

Study site

The experiment was carried out at the Sheep Breeding Station of Shahid Bahonar University of Kerman, Kerman, Southeast of Iran. The experiment was approved by the Department of Research and Experimental Animal Ethics Committee, Shahid Bahonar University of Kerman (file number 22143/granted on 2015/1/1). The experiment lasted 84 days, from January 2nd to March 25th.

Ensiling materials

Corn (variety 704) and common millet were obtained from the Plant Production Research Institute, and cultivated in May and harvested in mid-September (latitude 30°150N, longitude 57°010E, altitude 1755 m). The crops were chopped by a conventional forage chopper to the lengths of either 8 mm (short) or 16 mm (coarse). Chopped forages were ensiled without any additive in nylon bags with a size of 90×45 cm and stored at 20 - 25°C temperature for 45 days of ensiling.

Fleig point calculation

To determine the silage quality value, the Fleig point was calculated by the following equation (Denek and Can, 2006):

$$\text{Fleig point} = 220 + (2\times \%DM - 15) - 40 \times pH$$

Gas production

The *in vitro* gas production was measured according to the method described by Menke and Steingass (1988). Gas was produced in special syringes. The pressure of the gas was measured instead of the volume of gas produced. The volume of gas production was measured by using a regression equation between pressure and the volume of the produced gas (Theodorou et al., 1994). The apparatus was incubated at 39°C in the incubator; pressure of gas produced was recorded via digital barometer (Lutron model Pm-9100) at 2, 4, 8, 12, 16, 24, 48, 72 and 96 hours after incubation. The gas production parameters were estimated using the Fit curve by France et al. (1993) for estimating rumen degradability and gas production characteristics.

Experimental design, animals and diets

Four, 2-year-old Kermani rams (mean live weight of 39.2±3.1 kg) were assigned to four treatments in 2×2 factorial arrangement in Latin square design. Each period consisted of 21 days including 14 days of adaptation and 7 days for sampling. Animals were housed indoors under continuous light in individual metabolic crates (0.75 m × 1.5 m). The diets were iso-energetic and iso-nitrogenous (Table 1) consisting of 50% forage and 50% concentrates and fed as a total mixed rations (TMR) at 08:00 and 18:00 h each day. Animals had free access to fresh water throughout the study.

The silages were used in experimental diets (Table 1) as coarse corn silage (CCS); short corn silage (SCS); coarse millet silage (CMS) and short millet silage (SMS) diets.

Chemical composition, and nutrient intake and digestibility

Samples of feed ingredients, feed refusal and fecal samples were dried in a forced-air oven at 65°C for 48 h and then ground to a size of 1 mm before analysis.

Standard methods were used to measure the dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in feed, orts and fecal samples (AOAC, 2005). Dry matter intake (DMI) was recorded daily and manger contents, sampled at 0 and 24 h after feeding on d 15 to 19, were analyzed for DM, OM, CP and NDF. Intakes of DM, OM, CP and NDF were determined by subtracting the amount of offered and refused feedstuff. The total fecal collection method was used for calculation of DM and nutrient digestibility (Rymer, 2000).

Particle size distribution and peNDF

Feed particle size distribution was determined by dry sieving in four replicates using three sieves of Kononoff et al. (2003) and two sieves of Lammers et al. (1996) of Penn State Particle Separator (PSPS). The physical effective fiber (pef) values were determined as the proportion of DM retained on three sieves ($pef_{>1.18}$) or two sieves (pef>$_8$). The physically effective neutral detergent fiber (peNDF) was calculated by multiplying the NDF content of TMR on pef of three sieves (peNDF>$_{1.18}$) or two sieves (peNDF>$_8$). The geometric mean (GM) and the standard deviation of GM were calculated according to ASAE S424.1 (2002).

Urine sampling and nitrogen and protein production in the rumen

The output of urine produced was collected in special containers under metabolic cages on the sampling days of each period and a 20-mL subsample was stored at -20 °C. The subsamples were mixed and 20 mL of urine was collected for laboratory analysis at the end of each period. When the pH of samples was greater 3, 10% sulfuric acid was added to reduce the pH to below 3.

Microbial nitrogen and protein synthesis in the rumen, were calculated from purine derivative production in urine (Chen and Gomez, 1992) as:

$$Y=\frac{X\,(\frac{mmol}{day})\times 70}{0.116\times 0.83\times 1000}$$

In this equation, Y=synthesis of microbial nitrogen in the rumen (g/day), X= purine derivatives of microbial origin in urine (mmol/day).

Table 1. Ingredients and chemical composition of the experimental diets

ST[1]	Corn		Millet	
SPS[2]	Coarse	Short	Coarse	Short
Components (DM%)				
CCS[3]	40	-	-	-
SCS[4]	-	40	-	-
CMS[5]	-	-	40	-
SMS[6]	-	-	-	40
Alfalfa	10	10	10	10
Ground barley	23	23	23	23
Ground corn	5	3.5	-	3
Soybean meal	8	9.5	6	3
Wheat bran	12	12	19	19
Mineral/vitamin supplement[7]	0.5	0.05	0.05	0.5
Ground limestone	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Chemical composition (%) and metabolizable energy				
DM[8]	64.5	64.1	69.8	68.7
OM[9]	92.4	91.7	90.8	89.1
CP[10]	13.1	13.1	13.1	13.3
NDF[11]	40.2	39.1	41.2	40.7
ADF[12]	22.0	21.0	20.8	21.5
Ca (g)	6.5	6.4	6.4	6.3
P (g)	3.96	4.02	5.23	5.23
ME[13] (Mcal/kg DM)	2.32	2.35	2.33	2.31

[1]Sillage type. [2]Silage particle size. [3]Coarse corn silage. [4]Short corn silage. [5] Millet Silage. [6] short millet silage. [7]Per kilogram: 2.25 g Mn, 120 g Ca, 7.7 g Zn, 10 g P, 20.5 g Mg, 186 g Na, 1.25 g Fe, 3 g S, 1.25 Cu, 14 mg Co, 56 mg I, 10 mg Se, 52,00 IU of Vitamin A, 5000 IU of Vitamin D3, 150 IU of Vitamin E. [8]Dry matter. [9]Organic matter. [10]Crude protein. [11]Neutral detergent fiber. [12]Acid detergent fiber. [13]Metabolizable energy.

Measurement of the ruminal pH and ammonia nitrogen

Samples of ruminal fluid were collected via an esophageal tube at 0 (before feeding), 2, 4, 6, and 8 h after the morning feeding on the last day of each experimental period. The samples were strained through four layers of cheesecloth and the fluid pH was measured immediately by pH meter (Elmetron 103 CP). Ammonia-nitrogen (NH_3-N) was measured according to Broderick and Kang (1980).

Chewing behavior

Eating and ruminating activities, monitored visually over a 24 h period at d 21 of each experimental period, were recorded at 5-min intervals, and each activity was assumed to persist during the entire 5-min interval. Total time spent chewing was calculated as the total time spent eating and ruminating (Teimouri Yansari et al., 2004). Intake of OM, DM and NDF was used to calculate the eating, rumination and chewing activity per kilogram of OM, DM and NDF (Teimouri Yansari et al., 2004; Sharifi-Hosseini et al., 2012;).

Statistical analysis

Chemical composition and *in vitro* gas production data were analyzed by using the Proc GLM in SAS (2005, version. 9.1) according to the following model:

$$Y_{ijk} = \mu + \alpha_i + ß_j + (\alpha \times ß)_{ij} + e_{ijk}$$

where, Y_{ijk}=represents the observation, μ=total mean, α_i=effect of levels of silage particle size (SPS), β_j=effect of ST, $(\alpha \times \beta)_{ij}$=interaction effect of SPS by ST, e_{ijk} = random error. Data collected were analyzed using the GLM procedure in SAS (2005, version. 9.1) with significant Mean separation was performed by using the Tukey's test at 5% level.

To analyze the in vivo recorded data, the following model was used:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \gamma_k + \delta_L + e_{ijkl}$$

where, Y_{ijkl} = represents the observation, μ=total mean, α_i=effect of SPS, β_j=effect of ST, $(\alpha \times \beta)_{ij}$=interaction effect of SPS by TS, γ_k=random effect of male sheep, δ_L=effect of periods and e_{ijk}=random error. Data were analyzed using the Proc MIXED procedure in SAS (2005, version 9.1). The means were compared by the Tukey's test at 5% level.

Results

Silage characteristics

The DM of silages was affected by SPS and silage type (ST) and SPS × ST interaction, and it was higher in MS and short SPS (P<0.05, Table 2).

The concentration of CP, NDF, ADF, and pH were influenced by the SPS, ST, and their interaction. In both CS and MS, CP percentage was higher in short silage (P<0.05). The NDF and ADF contents were higher in CS and silages with short SPS compared with the MS and coarse silages, respectively. Silages with short particle size had lower NDF and ADF than the coarse silages (P<0.05). The pH was lower in CS and short silages than MS and coarse silages (P<0.05).

The Fleig point was higher in CS (P<0.05). The b value and gas production potential (a+b) were higher in CS than MS (P<0.05). The "a+b" values of gas kinetic parameters were lower in short SPS than coarse silage (P<0.05).

Table 2. Effect of silage type and silage particle size on chemical component (DM% bases), pH, Fleig point, and effect on gas kinetic parameters (n=5).

ST[1]	Corn		Millet		Main effects					P-value		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short	SEM[3]	ST	SPS	S×P[4]
DM[5]	23.9^{c}	23.3^{c}	39.5^{a}	34.3^{b}	23.6^{B}	36.9^{A}	31.7^{a}	28.3^{b}	0.003	<0.01	<0.01	<0.01
CP[6]	8.3^{b}	7.3^{c}	9.0^{b}	12.1^{a}	7.8^{B}	11.0^{A}	8.7^{b}	12.1^{a}	0.05	<0.01	<0.01	<0.01
NDF[7]	58.5^{a}	57.5^{b}	50.5^{c}	47.4^{d}	58.0^{A}	49.0^{B}	54.5^{a}	52.5^{b}	0.04	<0.01	<0.01	<0.01
ADF[8]	33.5^{a}	30.7^{c}	31.6^{b}	28.8^{d}	32.1^{A}	30.2^{B}	32.5^{a}	29.8^{b}	0.04	<0.01	<0.01	<0.01
ADL[9]	4.93	4.81	3.56	3.38	4.87^{A}	3.47^{B}	4.24	4.10	0.09	<0.05	0.25	0.65
pH	3.9^{c}	3.8^{d}	4.8^{a}	4.5^{b}	3.9^{B}	4.7^{A}	4.3^{a}	4.2^{b}	0.05	<0.01	<0.01	<0.01
Flieg points	99.6	98.7	92.0	93.5	99.1^{A}	92.8^{B}	95.8	96.1	1.14	<0.01	0.72	0.21
Gas kinetic parameters (mL/200mgDM)												
A[10]	50.4	41.8	41.9	31.9	46.1^{A}	36.9^{B}	46.1^{a}	36.9^{b}	2.26	<0.01	<0.01	0.75
(a+b[11])[12]	52.4	47.2	46.4	37.7	49.8^{A}	42.1^{B}	49.4^{a}	42.5^{b}	2.48	0.03	0.02	0.49
C[13]	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.004	0.66	0.96	0.42

$^{a,..c}$ Within row, means with common letters are not different (P>0.05).
[1]Silage type. [2]Silage particles size. [3] Standard error of the mean. [4]Interaction between ST and SPS. [5]Dry matter. [6]Crude protein. [7]Neutral detergent fiber. [8]Acid detergent fiber. [9]Acid detergent lignin. [10]Gas production at zero time. [11]Gas production from the insoluble part. [12]Gas production potential. [13]Gas production/unit time.

Particle size distribution and diet physical characteristics

The DM that remained on 19 and 8-mm sieves was higher in diets including coarse silages (Table 3; P<0.05), but the effect of ST was not significant. The DM that remained on 1.18 mm sieve was higher in diets containing short silage (P<0.05). The geometric mean and $peNDF_{>8}$ values were the highest in coarse SPS diets. The $peNDF_{>8}$ value was higher in MS than CS diets (P<0.05).

Feed intake and nutrient digestibility

The DM, OM, CP and NDF intakes were affected by SPS being higher in short SPS than coarse SPS diets (Table 4, P<0.05). The OM and NDF intakes were higher in CS diets than MS diets (P<0.05).

The DM, OM and NDF digestibility coefficients were affected by ST being higher in diets containing CS than MS (P<0.05). The SPS did not affect the digestibility of DM, OM, and NDF. The digestibility of crude protein was higher in short silage than coarse silage diets (P<0.05).

Ruminal pH

The mean of ruminal pH was influenced by the ST and being lower in diets containing CS than MS diets as well as the pH at the zero time (P<0.05) (Table 5).

Ruminal ammonia nitrogen

The ruminal fluid concentration of NH_3-N was not affected by the ST and the SPS at 0 and 2 hours after feeding. However, ruminal NH_3-N was the highest in CS diets at 4 hours after feeding. The concentration of NH_3-N was lower in short than coarse silages at 6 and 8 hours after feed consumption (P<0.05). Mean ruminal concentration of N-NH_3 was higher in CS and short SPS diets (P<0.01). The highest NH3-N mean concentration was found in CCS diet (P<0.05) (Table 6).

Table 3. Effect of silage type and silage particle size on particle size distribution and the physical effective factor and geometric mean of the experimental diets.

ST[1]	Corn		Millet		Main Effects					P-*value*		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short	SEM[9]	ST	SPS	S×P[10]
DM retained on sieves[4], % of total												
19 mm	23.1^{a}	12.0^{b}	25.1^{a}	14.3^{b}	17.3	19.6	24.8^{a}	13.3^{b}	1.63	0.21	<0.01	0.01
8 mm	33.0	29.4	41.3	35.1	32.2	37.4	37.4^{a}	32.2^{b}	5.71	0.35	0.01	0.05
1.18 mm	33.6	47.2	22.8	39.3	39.4	31.1	28.2^{b}	43.2^{a}	2.37	0.44	<0.01	0.24
pan	10.2	11.3	10.2	11.1	10. 8	10.9	10.2	11.5	2.78	0.55	0.27	0.93
GM[5] (mm)	8.25^{a}	6.15^{b}	7.45^{a}	5.44^{b}	7.20	6.44	7.8^{a}	5.79^{b}	6.44	0.46	0.04	0.03
SGM[6] (mm)	2.83	2.88	2.59	2.64	2.85	2.61	2.87	2.76	-	-	-	-
Physically effective NDF, % of DM												
peNDF$_{>8}$[7]	56.2^{b}	41.4^{d}	66.7^{a}	49.4^{c}	48.8^{B}	58.2^{A}	61.6^{a}	45.3^{b}	6.37	0.03	<0.01	0.04
peNDF$_{>1.18}$[8]	94.3	88.7	89.7	88.8	91.51	89.4	92.0	88.5	2.91	0.82	0.17	0.99

[a,..c] Within row, means with common letters are not different (P>0.05).
[1]Silage type. [2]Silage particles size. [3]Physical effective fiber. [4]Particle size distribution = material retained on each ASAE S424.1 sieve as % of DM (ASAE S424.1, 2002). [5]Geometric mean length as calculated by the ASAE (2002). [6]Standard deviation of geometric mean as calculated by ASAE (2002). [7]Physical effective NDF calculated as ration NDF multiplied by cumulative proportion of particles retained on 2 sieves (Lammers et al., 1996) of Penn State Particle Separator, respectively. [8]Physical effective NDF calculated as ration NDF multiplied by cumulative proportion of particles retained on 3 sieves (Kononoff et al., 2003b) of Penn State Particle Separator, respectively. [9]Standard error of the mean. [10]Interaction between SPS and ST.

Table 4. Effect of silage type and silage particle size on the intake and digestibility of nutrients in sheep

ST[1]	Corn		Millet		Main Effects					P-*value*		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short	SEM[7]	ST	SPS	S×P[8]
Nutrients intake (kg/day)												
DM[3]	1.61	1.75	1.56	1.69	1.68	1.63	1.59^{b}	1.72^{a}	0.03	0.20	0.01	0.90
OM[4]	1.49	1.60	1.30	1.40	1.55^{A}	1.35^{B}	1.41^{b}	1.50^{a}	0.02	<0.01	0.02	0.88
CP[5]	0.21	0.22	0.21	0.23	0.22	0.22	0.21^{b}	0.22^{a}	0.04	0.81	<0.01	0.49
NDF[6]	0.65	0.71	0.59	0.66	0.67^{A}	0.62^{B}	0.61^{b}	0.68^{a}	0.01	0.03	<0.01	0.65
Digestibility of nutrients (%)												
DM	68.3	72.2	59.3	63.3	70.3^{A}	61.3^{B}	63.8	67.7	2.02	<0.01	0.13	0.90
OM	71.8	75.0	59.3	62.4	73.4^{A}	61.1^{B}	63.8	67.8	2.12	<0.01	0.26	0.91
CP	70.4	77.3	71.1	72.6	73.8	71.8	70.7^{b}	75.0^{a}	1.98	0.36	0.01	0.49
NDF	56.2	61.2	38.3	41.7	58.7^{A}	42.5^{B}	47.7	53.9	3.19	<0.01	0.18	0.85

[a,..c] Within row, means with common letters are not different (P>0.05).
[1]Silage type. [2]Silage particles size. [3]Dry matter. [4]Organic matter. [5]Crude protein. [6]Neutral detergent fiber. [7]Standard error of the mean. [8]Interaction between SPS and ST.

Table 5. Effect of silage type and silage particle size on ruminal pH in different hours after morning feeding

ST[1]	Corn		Millet		Main effects					P-*value*		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short	SEM[3]	ST	SPS	S×P[4]
Time												
0	7.08	6.87	7.24	7.27	6.97^{A}	7.26^{B}	7.16	7.07	0.16	<0.01	0.20	0.10
2	6.48	6.49	6.67	6.52	6.48	6.59	6.57	6.51	0.14	0.55	0.71	0.64
4	6.38	6.52	6.48	6.68	6.45	6.58	6.43	6.58	0.09	0.32	0.20	0.79
6	6.74	6.43	6.64	6.68	6.59	6.66	6.59	6.66	0.14	0.58	0.34	0.29
8	6.96	6.80	6.93	6.99	6.88	6.96	6.95	6.89	0.10	0.56	0.67	0.40
Mean of ruminal pH	6.73	6.62	6.93	9.83	6.67^{B}	6.81^{A}	6.76	6.72	0.04	0.04	0.52	0.29

[a,..c] Within row, means with common letters are not different (P>0.05).
[1]Silage type. [2]Silage particles size. [3]Standard error of the mean. [4]Interaction between SPS and ST.

Purine derivatives and microbial protein synthesis in the rumen

The mean concentrations of allantoin, uric acid and total purine derivatives in urine were affected by the SPS (P<0.01) (Table 7). Allantoin, uric acid and total purine derivatives were the highest in fine SPS diets (P<0.05). Microbial protein (MP) synthesis was higher in short SPS than coarse SPS diets (P<0.05).

Feed intake behavior

Feeding and rumination activity (min/day) were not affected by ST and SPS and their interaction (Table 8), but chewing activity was affected by ST and SPS and was higher in MS and short SPS diets (P<0.05).

The duration time of eating per kilogram of DM, OM and NDF was not affected by SPS and ST. The duration time of rumination activity per kg of OM was affected by ST and was higher in MS diets (P<0.05). However, the duration time of rumination activity per kg of DM and NDF was not affected by SPS and NDF.

The duration time of chewing activity per kg of DM and OM was affected by ST, and was higher in MS diets than CS diets (*P*<0.05). The duration time of chewing activity per kg of DM was affected by SPS and was longer in coarse SPS diets (P<0.05).

Table 6. Effect of silage type and silage particle size on ruminal ammonia nitrogen at different hours after morning feeding (mg/dL)

ST[1]	Corn		Millet		Main effects				SEM[4]	P-*value*		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short		ST	SPS	S×P[5]
Time												
0	20.2	22.5	17.6	16.7	21.3	17.0	18.9	19.2	3.34	0.47	0.95	0.77
2	45.6	37.7	47.1	37.5	41.7	42.3	46. 6	37.6	5.20	0.93	0.27	0.91
4	37.7	31. 7	16.3	25.6	34.6[A]	20.5[B]	24.8	28.5	1.29	0.01	0.64	0.15
6	39.4[a]	10.0[c]	12.4[c]	20.4[b]	24.7	16.2	25.9[a]	15.0[b]	4.62	0.06	0.03	<0.01
8	39.2	13.2	21.5	15.5	26.2	18.6	30.5[a]	14.4[b]	6.73	0.13	<0.01	0.06
Mean N[3]	36.55[a]	25.6[b]	22.6[c]	23.4[c]	31.1[A]	23.0[B]	29.6[a]	24.5[b]	2.55	<0.01	<0.01	<0.01

[a,..c] Within row, means with common letters are not different (P>0.05).
[1] Silage type. [2] Silage particle size. [3]Ammonia nitrogen. [4]Standard error of the mean. [5]Interaction between SPS and ST.

Table 7. Effect of silage type and silage particle size on purine derivatives production (mmol/d) and synthesis of microbial protein in the rumen (g/day)

ST[1]	Corn		millet		Main effects				SEM[4]	P-*value*		
SPS[2]	Coarse	Short	Coarse	Short	Corn	Millet	Coarse	Short		ST	SPS	S×P[5]
Alantoin	6.25[b]	7.98[a]	4.51[c]	8.22[a]	7.11	6.37	5.38[b]	8.10[a]	0.82	0.41	<0.01	0.28
Uric acid	0.43[b]	0.86[a]	0.46[b]	0.89[a]	0.57	0.69	0.41[b]	0.84[a]	0.09	0.56	<0.01	0.96
Xanthan and Hypoxanthine	0.91	0.85	0.71	0.69	0.90	0.75	0.82	0.73	0.13	0.12	0.76	0.67
Total purine derivatives	7.62[b]	9.58[a]	5.68[c]	9.85[a]	8.60	7.76	6.65[b]	9.71[a]	0.73	0.40	0.01	0.29
MP[3]	38.7[b]	48.9[a]	27.1[b]	52.2[a]	39.7	44.8	33.0[b]	51.2[a]	5.07	0.38	0.01	0.28

[a,..c] Within row, means with common letters are not different (P>0.05).
[1] Silage type. [2]Silage particle size. [3]Microbial protein. [4]Standard error of the mean. [5]Interaction between SPS and ST.

Table 8. Effect of silage type and silage particle size on feeding behavior in sheep

ST[1]	Corn		Millet		Main effects				SEM[6]	P-*value*		
SPS[2]	Coarse	short	Coarse	Short	Corn	Millet	Coarse	Short		ST	SPS	S×P[7]
Feeding behavior (min/day)												
Eating	273	238	242	296	256	269	258	267	18.70	0.57	0.60	0.09
Rumination	326	238	310	331	282	320	318	285	45.31	0.27	0.33	0.13
Chewing	600[a]	477[c]	522[b]	627[a]	538[B]	590[A]	476[a]	552[b]	42.60	0.03	0.04	0.05
Eating activity per kg of .. (min)												
DM[3]	169	135	154	175	152	164	161	155	11.63	0.38	0.65	0.08
OM[4]	182	148	185	212	165	198	184	180	13.82	0.07	0.80	0.09
NDF[5]	175	169	146	195	172	171	161	182	12.93	0.91	0.20	0.12
Rumination activity per kg of .. (min)												
DM	199	136	199	195	167	199	201	165	28.69	0.16	0.11	0.21
OM	215	148	244	236	181[B]	240[A]	229	192	34.35	0.04	0.16	0.25
NDF	211	169	182	219	190	200	196	194	29.97	0.65	0.94	0.12
Chewing activity per kg of .. (min)												
DM	369[a]	271[b]	357[a]	370[a]	320[B]	363[A]	364[a]	320[b]	28.69	0.04	0.04	0.05
OM	398[b]	296[c]	430[ab]	448[a]	347[B]	439[A]	414	372	32.46	0.01	0.14	0.05
NDF	386	339	328	416	363	372	357	377	30.20	0.75	0.49	0.06

[a,..c] Within row, means with common letters are not different (P>0.05).
[1]Silage type. [2]Silage particles size. [3]Dry matter. [4]Organic matter. [5]Neutral detergent fiber. [6]Standard error of the mean. [7]Interaction between SPS and ST.

Discussion

Silage characteristics

It was expected that due to the higher evaporation, the DM in the short SPS might be higher, but a higher DM was recorded in coarse silages (Table 2). This is probably due to the reduced particle size in short SPS and increased aerobic respiration at early stage of ensiling. Therefore, the DM in silages was converted to H_2O and CO_2 leading to the decreased DM content (McDonald et al., 2011).

The higher CP in MS was consistent with the results of Brunette et al. (2014). However, Khan et al. (2010) reported higher CP content in CS compared with MS, thus it appears that MS quality was very low with its 5.2% protein content. The CP percentage was higher in short SPS. This is because pH and proteolysis decreased in short SPS. Therefore, lower levels of NH_3-N were produced and consequently there was more nitrogen in the form of protein (McDonald et al., 1991).

The NDF, ADF and ADL contents were higher in CS. These finding are inconsistent with Brunette et al. (2014) who reported that MS had a higher NDF content. The NDF and ADF in short silage were lower than coarse silage particles size. Yahaya et al. (2002) reported that acidic conditions can decrease NDF and ADF concentrations in silages. In this experiment, pH was lower in short than coarse silages; however, several studies reported that SPS did not affect the NDF and ADF concentrations (Soita et al., 2000; Maulfair and Heinrichs, 2013). In these experiments, short silage particles size was prepared by re-chopping the coarse CS and barley silage without any effect on the silage pH.

The pH was lowest in CS and short SPS. The higher DM in MS reduced microbial fermentation, resulting in lower acid production and higher pH (McDonald et al., 1991). Moreover, smaller SPS means more damage to plant tissues which can be fermented more efficiently by the lactic acid bacteria (McDonald et al., 2011). In silages with short particles size, large amount of manganese ions in the plant sap are used by the lactic acid bacteria (Raccach, 1985) which improves their proliferation conditions and lowers the pH of silage (McDonald et al., 1991).

The Fleig point was highest in CS; higher Fleig point means lower pH and higher DM in silage contents (Moselhy et al., 2015). According to Hosseini et al. (2017), pH is usually low in CS, because of high water-soluble carbohydrate content and low buffer capacity of corn forage. The Fleig point in the CS and MS (80-100), shows high quality of both silages (Denek and Can, 2006).

The b and a+b values were higher in CS. Starch content in CS is higher than MS (Brunette et al., 2014), and approximately 50% of energy value of whole corn plant comes from starch (Ferraretto and Shaver, 2012). There is linear relationship between gas production and starch degradation (Chai et al., 2004). The a+b values of gas production were lower in short SPS, which might be due to the higher lactic acid production in short silage. It should be noted that fermentation products in silage are not utilized by the rumen microbes (McDonald et al., 2011). Gas production values of CS were in agreement with Mould et al. (2000), who reported 53-56 mL gas/200mL OM produced after 96h incubation.

Particle size distribution and GM of diets

The coarse silage diets had higher DM remaining on 19 and 8-mm sieves. In the experiment of Maulfair et al. (2010), the amount of remained DM from coarse forage diets was higher on the upper sieves of 26.9 and 19 mm. The DM remained on 1.18 mm sieve was higher in diets with short SPS, because large amounts of DM passed through the upper sieves (Sharifi et al., 2012).

The GM was higher in CCS and CMS diets, because more DM remained on the upper sieves and the effect of these sieves were higher on the geometric mean (ASAE, 2002).

The $peNDF_{>8}$ value was highest in MS and coarse SPS diets, although among the same size of chopped corn and millet, longer size chopped millet forage had more DM remaining on the upper sieves. Corn and millet were chopped with the same sizes. Because of higher DM content, millet fodder had higher particles size when chopped by chopper. Therefore, MS diets had higher $peNDF_{>8}$ than CS diets. This might be due to high lignin content and lignification in millet (Messman et al., 1992; Brunette et al., 2014).

DM and nutrient intake and digestibility

The nutrient intakes were highest in short SPS diets. Forages with low specific gravity have higher fill effect than forages with higher specific gravity (Wattiaux, 1990). Functional specific gravity increased as forage size decreased (Teimouri Yansari et al., 2004). Diets containing coarse SPS have higher rumen fill effect, because of lower rumen passage rate. Forage particle size is responsible for 59% of the variation in the average retention time in the rumen of sheep (Kaske and Engelhardt, 1990). Consequently, the nutrient intakes were limited due to expanded rumen (Kononoff and Heinrichs, 2003). It was reported by Nasrollahi et al. (2014), that in cows which were fed with high quality forage, reducing particle size is a useful tool for increasing DMI.

The OM and NDF intakes were highest in CS diets, because OM and NDF digestibility was higher in these diets (Table 4). Combs (2015) reported that 1% change *in vitro* or *in situ* NDF digestibility was correlated with a 0.17 kg increase in voluntary DMI in cows.

The DM and OM digestibility were highest in CS diets. Brunette et al. (2014) found higher starch content in CS than MS diets. Starch concentration is a major source of energy in CS and contribute 50- 70% of digestible OM (Andrae et al., 2001). Khan et al. (2010) reported that DM digestibility of the 100% corn silage and corn silage plus 50% concentrate diets were significantly higher than those of sorghum silage and MS. The results of this study were consistent with the result of Ward et al. (2001) who reported that CS had the highest DM digestibility, followed by sorghum silage and MS.

The SPS did not affect the nutrient digestibility. Probably, the shorter silage particle size had an increasing effect on the ruminal passage rate (k_p) which decreased the access of ruminal microorganisms to the nutrients, resulting in decreased nutrient digestibility (Clark and Armentano, 2002). However, digestion rate did not change through digestive tract as reduced digestion in the rumen was compensated by increased digestion in the lower digestive tract (Knapp et al., 2014).

The CP digestibility was highest in short silage diets. This might be due to the reduction in nitrogen recycling in the rumen as protozoa mass decreases in low-peNDF diets (Yang and Beauchemin, 2006). Yang and Beauchemin (2004) found that CP digestibility in the rumen and digestive tract was higher in short silage compared with coarse silage.

The NDF digestibility was highest in CS diets. Results of this experiment were consistent with Messman et al. (1992) who also reported that NDF in MS was less digestible than CS because of higher lignification.

Ruminal fluid pH

The ruminal pH at the zero time and the mean of pH were lowest in CS. High starch content in CS has a positive effect on reducing ruminal pH (Brunette et al., 2014; MacDonald et al., 2011). Also, rapidly fermentable carbohydrates had a greater effect on reducing pH in goat (Zhao et al., 2010). High concentrations of starch in CS diets led to more volatile fatty acids production and decreased the ruminal fluid pH (Ruppert et al., 2003). Due to decreased salivation, it was expected that the ruminal fluid pH would decrease in short SPS diets (Teimouri Yansari et al., 2004), but Zebeli et al. (2010), suggested that ruminal pH cannot be predicted only by using physical properties of the diets while fermentable carbohydrates (CHO) have a greater effect on ruminal pH.

Ruminal fiuid NH_3-N

The NH_3-N concentration in the ruminal fluid was not affected by the silage type and the SPS at 0 and 2 hours after feeding, which might be due to fermentation lag time (Griffith et al., 2016). The MS diets had the lowest mean ruminal NH3-N and NH3-N concentration at 4 hours after feeding. The CS diets fermented and produce lower fermented energy in the rumen, because CS produced higher ruminal lactic acid (CS had lower pH value). The CS produced less gas compared to MS (Table 2) because silage fermentation products are not fermented by the rumen microorganism (MacDonald et al., 2011).

The concentration of NH_3-N was lowest in short SPS at 6 and 8 hours after feed intake ($P<0.05$). Soita et al. (2000) found that by reducing particle size of barley silage, starch degradability and ammonia utilization for microbial protein synthesis were increased. When protein degradation rate exceeds the rate of CHO fermentation, large quantities of N can be lost as ammonia; conversely, when the rate of CHO fermentation exceeds protein degradation rate, microbial protein synthesis increases (Nocek and Russell, 1988). Mean ruminal concentration of N-NH_3 was highest in CS diets. The highest NH3-N mean concentration was in CCS diet, because of higher concentration of NDF and ADF in this diet.

Purine derivatives

Allantoin, uric acid and total purine derivatives were highest in short SPS diets. The Increase in alanthine can be attributed to increased feed intake and higher protein synthesis (Chen et al., 1996). In the current experiment, the DMI was highest in short SPS diets; as a result, protein intake was higher in short SPS diets. Increasing CP intake and reducing the peNDF in short SPS diets increased the purine derivatives in the urine (Broderick, 2003). Rumen MP synthesis depends significantly on the synchronization of access to carbohydrates and nitrogen in the rumen (Stokes and Hoover, 1991).

Microbial protein synthesis

Microbial protein (MP) synthesis was higher in short SPS diets. The MP synthesis increased in the rams fed with diets low in peNFD (Yang and Beauchemin, 2006). The rate of protein degradation increased in the rumen by decreasing silage particles size (Yang and Beauchemin, 2004). Therefore, higher MP synthesis in short SPS could be related to more ammonia production in the rumen (Maulfair and Heinrichs, 2013). Starch degradability and ammonia production increased by reducing the silage particles size simultaneously (Soita et al., 2000; Yang and Beauchemin, 2007). Rumen MP synthesis also depends significantly on the synchronization of access to carbohydrates and nitrogen in the rumen (Stokes and Hoover, 1991).

Feed intake behavior

Chewing activity was higher in MS and short SPS diets. The $peNDF_{>8}$ and GM were higher in coarse SPS and MS diets (Table 3). Forage particle size impacts on the feed intake, chewing activity, passage rate, rumen fermentation and acid production in the rumen (Taffaje et al., 2007). Increasing the dietary peNDF stimulated the chewing activity and improved ruminal PH in goats fed coarse alfalfa (Zhao et al., 2010). By reducing the forage particle size, the peNDF and GM decreased. However, decreasing the forage particle size increased the DMI and chewing activity increased in Zel sheep (Sharifi et al., 2012).

The duration time of rumination activity per kg of OM was highest in MS diets. Krause et al. (2002) suggested that forages could stimulate rumination, and in this experiment the $peNDF_{>8}$ was higher in MS diets (Table 3), thus MS had higher effect on rumination. Reducing $peNDF_{>8}$, resulted in lower rumination (Teimouri Yansari et al., 2004).

The duration time of chewing activity per kg of DM and OM was longer in MS diets. The $peNDF_{>8}$ was higher in MS diets (Table 3). Diets with higher peNDF affect chewing activity and rumen buffering (Zebeli et al., 2012). Teimouri Yansari et al., (2004) reported that reducing alfalfa particle size decreased rumination and total chewing activity per daily intake $peNDF_{>8}$ (kg) in cows. However, longer chewing activity per unit of feed was measured in sheep when compared to heifers (De Boever et al., 1990). The DM, OM and NDF digestibility was highest in CS diets (Table 3). The increased digestibility of NDF may cause more fragility of the cell wall and reduce the chewing activity (Zebeli et al., 2012).

The duration time of chewing activity per kg of DM was longer in coarse SPS diets. The $peNDF_{>8}$ and GM were higher in coarse SPS diets (Table 3), resulting in increased chewing activity (Sharifi et al., 2012). A short SPS may adversely affect stratification of ruminal digesta, providing less stimuli for chewing activity and ruminal contractions (Zebeli et al., 2010). The chewing behavior of cows and sheep reacts differently to forage quality, being longer in sheep especially on bad quality forages (De Boever et al., 1990). The digestibility of forages plays an important role in cows (Zebeli et al., 2012), sheep, and goats compared to dietary peNDF (Jalali et al., 2012).

Conclusion

The results of this experiment showed that millet silage can be a good source of forage, and short millet silage can provide more energy for small ruminants. Although millet silage may not meet 6 the energy requirements of ruminants, but when fed along with better quality forages it can be a good supplier of the required physical effective NDF.

Acknowledgment

This research was done with the financial support of Research and Technology Institute of Plant Production, Shahid Bahonar University of Kerman, Kerman, Iran.

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