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Effects of urea and molasses supplementation on chemical composition, protein fractionation and fermentation characteristics of sweet sorghum and bagasse silages as alternative silage crop compared with maize silage in the arid areas

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Abstract

This study was planned to determine the chemical composition, protein fractionation and fermentation characteristics of sweet sorghum and sweet sorghum bagasse silages treated with urea and molasses. Treatments were maize silage (MS), sweet sorghum silage (SS), sweet sorghum bagasse silage (BS) and/or urea and molasses supplemented (10 and 50 g/kg dry matter (DM) basis, respectively) SS and BS. Triplicate silage samples were prepared for each treatment in laboratory silos. Fresh sweet sorghum and its bagasse had greater (P<0.01) DM and NFC, but lower (P<0.01) CP and a NDF concentrations compared with maize. After 90 d of ensiling, similar results were found in produced silages. The SS and BS had lower protein "A" fraction but greater protein "C" fraction (P<0.01) compared with MS. Treating of SS and BS with urea or molasses resulted in an increased (P<0.05) in pH. Orthogonal comparisons showed adding urea increased CP, lactate, ammonia nitrogen (NH_3-N) concentrations, and pH value but decreased NFC (P<0.01). Adding molasses to SS and BS silages increased DM, WSC, NFC, pH (P<0.01) and *in vitro* digestibility of DM (P<0.05) while caused a decrease in a NDF, ADF, acetate (P<0.01) and ethanol (P<0.05). Adding of urea or urea plus molasses to SS and BB resulted an increase in protein "A" fraction (P<0.01) but a decrease in protein "C" fraction (P<0.01). Therefore, simultaneous application of urea and molasses improved the nutritional quality, DM digestibility, protein fractionations and fermentation characteristics of SS and BS and offered a potential alternative silage crop compared with maize in the arid areas.

Keywords: Additives; fermentation characteristics; protein fractionation; sweet sorghum; sweet sorghum bagasse

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Introduction

Sweet sorghum (*Sorghum bicolor* var. saccharatum) is a C_4 plant with high photosynthetic activity, resistant to drought and salinity (Almodares et al., 2008), contains a high level of energy (Negro et al., 1999) and can be cultivated in most tropical areas. Also, sweet sorghum stem juice can be used for production of ethanol as a bio fuel (Almodares and Hadi, 2009). There has been a growing tendency to produce ethanol from sweet sorghum juice in Iran. Therefore there is a concern about the disposal of

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the remaining sorghum bagasse (Filya, 2003). Sorghum bagasse (like stem juice) can be used for bio fuel production however; it is not an economically viable process so far (Drapcho et al., 2008). Therefore, in this study it was attempted to preserve this residue for long term and also produce potentially usable feed for ruminants.

Ensiling is a suitable method for forage conservation and is aimed at minimizing nutrient wastage by enhancing the growth of lactic acid producing bacteria (Baytok et al., 2005). However, inadequate crude protein in sorghum (55 to 90 g per kg DM) results in long fermentation time (Marrero et al., 2000) which may increase the temperature in the lower layers of the silage. Urea can be used to increase nitrogen concentration and improve the fermentation quality of the sorghum forage (Filya, 2001). Since soluble proteins could not be utilised optimally in the absence of adequate water soluble carbohydrates (WSC). Molasses, a source of WSC, is often used along with urea to help preventing silage instability (Jaurena and Pichard, 2001). Also, molasses prevents increase in silage temperature and poor aerobic stability of produced silage (Soderholm et al., 1998). Molasses has also been added to the silages to increase dry matter (DM) concentration, fermentation rate and production of lactic acid (McDonald et al., 1991).

Because the protein content of forages, silages or grains used in animal feeding are sometimes inadequate to meet the needs of the animal, protein supplements (e.g., adding urea in silages) become essential. Consequently, analysis for protein fractions or crude protein in a feed sample or silages is important. Also, there are very few reports about the sweet sorghum bagasse silage and its quality. However, the effects of additives such as urea and molasses on sweet sorghum and sorghum bagasse silages composition, fermentation characteristics and CNCPS protein fractions have not been investigated before. Therefore the present study was performed to investigate the quality of ensiled sweet sorghum and its bagasse and the effects of supplementation of urea and/or molasses on their quality in central Iran condition.

Materials and Methods

Plant material

Sweet sorghum (*Sorghum bicolor* var. saccharatum) was planted on 5 June 2011 in Isfahan University Research Farm and harvested after 120 d having a mean DM concentration of 331 g/kg fresh weight. The study location, crop management and fertilizers adding for sweet sorghum were the same as mentioned by Zafari Naeini et al. (2014). Maize was harvested after 70 d (early dent) at a mean DM concentration of 177 g/kg fresh weight which is a rather common practice in Iran. For maize, there was 16 cm distance between the bushes in a row and 76 cm distance between the rows and plant population was 70 to 80 thousands/ha. The plants were cut 15-20 cm from ground level. About 100 kg of maize forage and 200 kg of sweet sorghum forage were harvested and the materials were randomly divided into different batches needed for the preparation of treatments. To obtain sorghum bagasse, the grain clusters were separated by hand and the leaves by a special apparatus. The resulted stems were extracted using an apparatus having two pairs of rollers to reduce the weight by 200±20 g/kg fresh weight. Extracted stems along with separated leaves were chopped into 2-3 cm pieces. The same chaffing process was performed for whole maize and sweet sorghum forages. Sweet sorghum and maize silages were prepared from whole plants including stems, seeds and leaves.

Ensiling procedure

Whole sorghum and maize plants and sorghum bagasse were ensiled in PVC cylindrical shape containers with 4.0±0.2 kg capacity (50 cm height × 16 cm diameter).The density was 521±62.5, 543±48.5 and 451±29.0 kg/m^3 for fresh maize, sweet sorghum and sorghum bagasse forages, respectively. Urea and/or molasses (10 and 50 g/kg on DM basis, respectively) were added to the silage batches prior to filling whenever appropriate. To ensure precise mixing of urea and molasses to the plant material, first a small portion of the material were mixed with urea or molasses and then the portion was mixed with whole batch thoroughly. The laboratory silos had a 2-cm layer of sand in the bottom to help drainage process. A tap and hose were attached to the bottom of the silos to drain the effluent. After filling the silos, plant material were pressed using a pressing apparatus to ensure expelling of the air. The silos were closed tightly and the lids were lubricated with oil to be sealed effectively. The laboratory silos were placed in a dark room with average temperature of 18°C until opening after 90 d of preservation.

Treatments

The experimental treatments were as follow: 1) maize silage (MS), 2) sweet sorghum silage (SS), 3) sweet sorghum silage plus urea (SSU), 4) sweet sorghum silage plus urea and molasses (SSUM), 5) sweet sorghum bagasse silage (BS), 6) sweet sorghum bagasse silage plus urea (BSU), 7) sweet sorghum bagasse silage plus urea and molasses (BSUM). Three replicates were used for each treatment and in total twenty one laboratory silos were used in this experiment.

Sampling and chemical analysis of fresh and ensiled forages

Fresh forages

After chopping, 500 g of fresh forage was dried at 55°C for 48 h in triplicate for each treatment and then the dried material was ground to pass a 1 mm screen and stored in dark vacuum plastic bags at room temperature (20±2°C) for chemical analysis and *in vitro* incubation. The silages were evaluated after 90 d of ensiling. Before evaluation, 5 cm from the top and bottom ends of the silage in each silo were discarded and the remaining material was mixed thoroughly to ensure uniformity. Then a 2 kg fresh sample was transferred into vacuum plastic bags and frozen at -20°C for subsequent analysis.

Chemical analysis

A 30 g sample of fresh silage was mixed with 270 ml distilled water (1:9 ratio) and blended using a kitchen blender for 50 to 60 seconds at high speed. The extract was then filtered through four layers of cheese cloth and the pH was determined using a digital pH meter (Metrohm 744, Switzerland). Some of the extract was stored at -20°C until analyzed for acetic acid, lactic acid, ethanol and NH_3-N.

The frozen silage samples were oven-dried at 55°C for 48 h and ground through a 1 mm sieve for determination of chemical composition. The ether extract (EE), crude protein (CP) and ash were measured according to AOAC (1999) and the NFC was calculated (g/kg DM) using the following formula (Ishler and Varga, 2001):

NFC=1000-[ash+EE+CP+aNDF-NDIP]

Where NDIP is neutral detergent insoluble protein. The WSC was measured by phenol-sulfuric acid method (Masuko et al., 2005). The UV absorption was recorded at 470 nm wavelength using a spectrophotometer (Jasco V-570 UV/Vis/NIR spectrophotometer, Japan). Ethanol, propionic, butyric and acetic acid were measured by gas chromatography (Crompak, Model CP 9002, The Netherlands) as described by Playne (1985). The determination of lactic acid was carried out by high-performance liquid chromatography (HPLC) method developed by Megias et al. (1993). Ammonia-N (NH_3-N) was measured (Kjeltec Auto 1030 Analyzer, Sweden) in 50 ml of fresh silage extracts (without digestion) filtered through what man filter paper #1 (Filya, 2003). The fibre sections, NDF assayed with amylase and expressed inclusive of residual ash (aNDF) and acid detergent fibre (ADF) were measured using heat-resistant alpha-amylase and sodium sulphite (for starch and protein degradation, respectively) according to Van Soest et al. (1991) and acid detergent lignin (ADL) by hydrolysis method using 720 g/kg sulphuric acid (Van Soest and Wine, 1968).

Determination of *in vitro* digestibility of DM and ADF

In order to determine *in vitro* digestibility of DM (IVD_{DM}) and ADF (IVD_{ADF}), 0.5 g of dried silages samples, ground through a 1 mm sieve, were transferred into heat sealed F57 filter bags of Ankom and were incubated along with four empty bags as blanks. The buffer solutions A and B were prepared according to the instruction for Ankom Daisy^{II} Incubators (Ankom Technology, Macedon, NY, USA). An equal volume of the rumen fluid was obtained from 3 non-lactating Holstein cows (750.0±10 kg, consuming a total mixed ration) about 4 h after morning feeding and mixed. A maintenance ration (AFRC, 1992) was fed in equal portions two times per d (07:00 and 19:00) consisting of 490 g/kg silage (1:1 MS:SS), 100 g/kg concentrate (containing 5 g/kg urea), 400 g/kg chopped alfalfa and 10 g/kg molasses. The rumen fluid was immediately transported to the laboratory in a carbon dioxide flask and mixed using a kitchen blender for 30-60 seconds under anaerobic conditions (presence of CO_2). The fluid was then filtered through four layers of cheesecloth. Each Ankom jar contained 400 ml filtered rumen fluid, 25 bags, 266 ml B solution (15 g Na_2CO_3, 1 g $Na_2S.9H_2O$ per litre), 1330 ml A solution (10 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.1 g $CaCl_2.2H_2O$ and 0.5 g urea per litre)at pH=6.8. The jars were then placed in the Ankom Daisy^{II} device for 48 h at 39.5°C. At completion of incubation, the jars were removed and the fluid was drained. The bags were rinsed thoroughly with cold tap water with minimal mechanical agitation until the water was clear. The rinsed bags were transferred into the Ankom^{200} Fibre Analyzer, aNDF was determined based on the ANKOM protocol and the aNDF weight (W_3) was recorded. The bags were dried at 60°C for 48 h and IVD_{DM} was calculated as:

$$IVD_{DM}\ (g/kg\ DM) = 1000\{1-[W_3-(W_1\times C_1)]/(W_2\times DM)\}\times 1000$$

Where W_1 is the bag tare weight, W_2 is the sample weight, W_3 is the final bag weight after *in vitro* and sequential neutral detergent solution treatment and C_1 is the blank correction factor (final oven-dried weight/original blank bag weight). The following equation was used for determination of final ADF (ADF_{Final}) after 48 h of incubation in Ankom Daisy^{II} and washing with ADF solution in Ankom^{200} Fibre Analyzer:

$$ADF_{Final}(g/kg\ DM) = \{[W_4-(W_1\times C_1)]/(W_2\times DM)\}\times 1000$$

Where W_1, W_2 and C_1 have been previously described and W_4 is the final bag weight after *in vitro* and sequential acid detergent solution treatment. *In vitro* digestibility of ADF was calculated using the following equation:

IVD_{ADF} (g/kg total ADF) = $\{(A_1-A_2)/A_1\}\times1000$

Where: A_1 and A_2 are the primary and final ADF (g/kg DM), respectively.

Determination of the protein fractions

Protein fractionation was performed as described by Licitra et al. (1996). Non-protein nitrogen (A fraction) was calculated as the difference between the sample nitrogen content and precipitated true protein nitrogen. The B_1 fraction was estimated using borate-phosphate buffer and sodium azide solution. The NDIP and acid-detergent insoluble protein (ADIP or C fraction) were measured using neutral detergent and acid detergent solutions, respectively. The B_3 fraction was calculated by subtracting the amount of protein remaining in the sample washed with neutral detergentand protein remaining in the sample washed with acid detergent. TheB_2 fraction was calculated as CP – (A+B_1+B_3+C).

Statistical analysis

This experiment was done as a completely randomized design with seven treatments and three replicates. The data were analyzed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2003) based on the following model:

$Y_{ij}=\mu + T_i + e_{ij}$

Where μ is the overall mean for each parameter, T_i is treatment effect (i = 1–7) and e_{ij} is the residual. Percentage data were transformed into Arcsin before analysis and then reconverted to original unit for showing in Tables. The effects of urea (SS and BS *versus* SSU and BSU), molasses (SSU and BSU *versus* SSUM and BSUM) or urea plus molasses (SS and BS *versus* SSUM and BSUM) were assessed using orthogonal comparisons.

Results

Fresh forages

Sorghum forage had higher DM, WSC and NFC (P<0.05) but lower CP, EE, aNDF and ADF concentrations (P<0.01) compared with the maize forage (Table 1). Sorghum bagasse had higher DM, aNDF and ADF but lower CP and NFC concentrations than the sorghum forage. There were no differences in the concentrations of ADL, ash and IVD_{DM} (Table 1) and protein A fraction (Table 2) between whole sweet sorghum, maize forages, and sweet sorghum bagasse (P>0.05). Fraction B_1 was greater in bagasse compared with maize and fraction B_2 was greater in maize forage than in sorghum and its bagasse. B_3 fraction was greater in bagasse compared with maize and sorghum forages (P<0.01). Fraction C was the highest (P<0.01) in sorghum plant, medium in sorghum bagasse and the lowest in maize forage.

Table 1: Chemical composition (g/kg DM) of fresh maize forage, sweet sorghum forage and sweet sorghum bagasse

Parameters	Fresh material			SEM[1]	*P* value
	Maize	Sorghum	Sorghum Bagasse		
DM	177[c]	331[b]	362[a]	2.2	**
Ash	62	59	60	3.4	ns
CP	88[a]	56[b]	51[c]	2.4	**
EE	42[a]	30[b]	26[b]	2.9	**
WSC	94[b]	137[a]	152[a]	8.8	**
NDF	526[a]	447[c]	491[b]	6.1	**
ADF	263[a]	213[b]	258[a]	4.1	**
ADL	81	82	97	4.7	ns
NFC	310[c]	432[a]	394[b]	7.9	**
IVD_{DM}[2] (g/kg)	700	717	715	8.5	ns
$IVD_{(ADF)}$[3] (g/kg)	343[a]	243[b]	266[b]	14.9	**

[a–c]Within each row, means with the same superscript are not significantly different; [**]Significant difference at P<0.01, [ns]non-significant difference; [1] Standard error of the means for n=9 measurements; [2]*In vitro* digestibility of Dry matter(IVD; g/kg DM); [3]*In vitro* digestibility of Acid detergent fibre (ADF; g/kg of total ADF)

Table 2: Protein fractions (g/kg of crude protein) of fresh maize forage, sweet sorghum forage and sweet sorghum bagasse based on CNCPS method

Protein fractions[1]	Fresh material			SEM[2]	*P* value
	Maize	Sorghum	Sorghum Bagasse		
A	409	406	377	10.1	ns
B_1	25[b]	45[ab]	83[a]	29.7	*
B_2	261[a]	113[b]	107[b]	29.5	**
B_3	182[b]	193[b]	233[a]	7.2	**
C	122[c]	238[a]	195[b]	4.7	**

[a–c]Within each row, means with the same superscript(s)are not significantly different; [*]Significant difference at P<0.05, [**]Significant difference at P<0.01, [ns]non-significant difference; [1]A: Non protein nitrogen (NPN), B_1: buffer soluble protein, B_2: protein with medium degradation rate, B_3: protein insoluble in neutral detergent but soluble in acid detergent, C: protein insoluble in acid detergent; [2] Standard error of the means for n=9 measurements

Chemical composition of silages

The chemical composition of silages at 90 d of ensiling is shown in Table 3. Sweet sorghum silage (SS) and sweet sorghum bagasse silage (BS) had higher DM concentrations compared with MS (P<0.01). Urea, as an additive, increased CP but decreased the NFC concentrations of the silages (P<0.01). There were no significant differences (P>0.05) in ADL and EE concentrations among silages. Adding molasses decreased NDF and ADF concentrations (P<0.01) while increased DM, WSC, NFC and ash concentrations (P<0.01). Addition of both urea and molasses increased

Table 3: Chemical composition (g/kg DM) of maize, sorghum and sorghum bagasse silages with or without additives after 90 days of ensiling

Parameters	Silages[1]								Orthogonal contrasts				
	MS	SS	SSU	SSUM	BS	BSU	BSUM	SEM[2]	MS *vs.* SS and BS[3]	SS *vs.* BS[4]	Urea[5]	Molasses[6]	Urea + Molasses[7]
DM	203^{e}	342^{d}	364^{cd}	397^{b}	395^{b}	382^{bc}	422^{a}	5.1	**	**	ns	**	**
Ash	76^{a}	57^{b}	59^{b}	69^{ab}	70^{ab}	59^{b}	71^{ab}	3.1	**	*	ns	**	ns
CP	83^{a}	53^{c}	74^{ab}	68^{b}	41^{d}	74^{ab}	74^{ab}	2.2	**	**	**	ns	**
EE	33	27	29	27	23	25	27	2.8	*	ns	ns	ns	ns
WSC	41^{b}	40^{b}	42^{b}	117^{a}	39^{b}	57^{b}	63^{b}	5.5	ns	ns	ns	**	**
NDF	502^{a}	470^{ab}	450^{b}	403^{c}	470^{ab}	499^{a}	443^{bc}	8.3	**	ns	ns	**	**
ADF	272^{a}	254^{abc}	240^{bcd}	217^{d}	260^{abc}	264^{ab}	236^{cd}	5.4	**	ns	ns	**	**
ADL	79	83	82	71	96	88	78	8.8	ns	ns	ns	ns	ns
NFC	306^{c}	393^{ab}	388^{b}	433^{a}	395^{ab}	343^{c}	384^{b}	8.4	**	ns	**	**	ns
IVD_{DM}[8] (g/kg)	706^{ab}	686^{ab}	707^{ab}	756^{a}	662^{b}	677^{b}	719^{ab}	16.1	ns	ns	ns	*	**
$IVD_{(ADF)}$[9] (g/kg)	336	347	295	335	302	290	346	34.1	ns	ns	ns	ns	ns
Effluent[11]	36^{ab}	45^{a}	50^{a}	58^{a}	26^{ab}	17^{b}	24^{ab}	6.6	ns	*	ns	ns	ns

$^{a–c}$ Within each row, means with the same superscript(s) are not significantly different; *Significant difference at P<0.05, ** Significant difference at P<0.01, ^{ns} non-significant difference; [1]Maize silage (MS), Sweet sorghum silage (SS), Sweet sorghum silage treated with urea (SSU), Sweet sorghum silage treated with urea and molasses (SSUM), Sweet sorghum bagasse silage (BS), Sweet sorghum bagasse silage treated with urea (BSU), Sweet sorghum bagasse silage treated with urea and molasses (BSUM); [2]Standard error of the means for n=21 measurements; [3]Orthogonal comparison of MS *vs.* SS and BS; [4]Orthogonal comparison of SS *vs.* BS; [5]Orthogonal comparison of SSU and BSU *vs.* SS and BS *i.e.* effect of urea; [6]Orthogonal comparison of SSUM and BSUM *vs.* SSU and BSU *i.e.* effect of molasses; [7]Orthogonal comparison of SSUM and BSUM *vs.* SS and BS *i.e.* effect of urea plus molasses; [8]*In vitro* digestibility of dry matter (IVD; g/kg DM); [10]*In vitro* digestibility of Acid detergent fibre(ADF; g/kg of total ADF); [11]g/kg of fresh matter

DM, WSC (only for SSUM silage) and CP concentrations of the silages compared with the silages without any additive. The least effluent (P<0.01) was observed for bagasse silages.

Fermentation characteristics of silages

Fermentation characteristics of silages after 90 d of ensiling are shown in Table 4. All silages had pH values lower than 4.0. The pH was not different between MS, SS and BS silages. However, SSUM and BSUM silages had the highest pH values compared with other silages (P<0.05). There was no significant difference between silages in NH_3-N/total N (NH_3-N/N) concentration. Adding urea or urea plus molasses increased NH_3-N (P<0.01) concentration compared with their respective control silages, where the concentrations of ethanol, lactic acid and acetic acid were higher in MS than other silages (P<0.01). Adding urea plus molasses reduced ethanol and acetate (P<0.05) while increased lactate concentration (P<0.01). Propionic and butyric acid peaks were not detected in any of the silages.

Protein fractionation of silages

Characteristics of protein fractions based on CNCPS at d 90 of ensiling are shown in Table 5. Urea-treated silages had the highest protein A fraction (P<0.01). Fractions B_1 and B_3 values were not different among the silages (P>0.05). Fraction C value was not different among MS, SSU, SSUM, BSU and BSUM, while the highest C value was found in SS and BS silages. Urea treatment decreased the B_2 and C fractions (P<0.01).

Discussion

The low DM concentration of maize plant (177 g/kg DM; Table 1) might be due to the early stage of harvesting which resulted in low silage DM concentration of MS (203 g/kg DM; Table 3). Higher IVD_{ADF} of fresh maize compared with sorghum (100 g/kg difference) arising primarily from earlier maturity of maize compared with sorghum during the harvest time. As a result, in spite of higher ADF concentration of fresh maize (50 g/kg DM difference) compared with sorghum forage, no significant difference was found between fresh maize and sorghum *in vitro* digestibility of DM.

Sweet sorghum bagasse in the current study had rather different chemical composition than those reported in the literature. When sweet sorghum stalks were pressed by rollers to extract stem juice, fibrous bagasse remains which contained 270 to 480 g/kg cellulose, 190 to 240 g/kg hemicelluloses, and 90 to 320 g/kg lignin (Kim and Day, 2011; Cunningham et al., 1986). In a study by Anandan et al. (2012), sweet sorghum bagasse with leaf residues had lower CP (40 g/kg) and higher NDF (690 g/kg) contents, but lower IVD_{DM} (543 g/kg DM) compared with our results. The discrepancy in chemical composition of sweet sorghum

Table 4: Fermentation characteristics (g/kg DM) of maize, sorghum and sorghum bagasse silages with or without additives after 90 days of ensiling

	Silages[1]								Orthogonal contrasts				
Parameters	MS	SS	SSU	SSUM	BS	BSU	BSUM	SEM[2]	MS *vs.* SS and BS[3]	SS *vs.* BS[4]	Urea[5]	Molasses[6]	Urea + Molasses[7]
pH	3.79^{c}	3.74^{c}	3.80^{bc}	3.86^{ab}	3.75^{c}	3.79^{c}	3.91^{a}	0.014	ns	ns	**	**	**
Lactate	72.0^{a}	26.0^{c}	36.0^{b}	33.0^{b}	25.0^{c}	27.0^{c}	27.0^{c}	0.88	**	ns	**	ns	**
Acetate	21.4^{a}	11.3^{bc}	11.7^{bc}	7.7^{c}	10.4^{bc}	12.7^{b}	9.3^{bc}	0.97	**	ns	ns	**	*
Ethanol	19.5^{a}	2.1^{b}	2.1^{b}	1.3^{b}	1.9^{b}	2.1^{b}	1.5^{b}	0.77	**	ns	ns	*	*
Ammonia-N	1.72^{a}	0.96^{b}	1.47^{ab}	2.03^{a}	0.99^{b}	2.11^{a}	2.05^{a}	0.34	**	ns	**	ns	**
Ammonia-N/N (%)	12.9	11.3	12.7	18.8	15.8	17.9	17.3	1.72	ns	ns	ns	ns	*

$^{a–c}$Within each row, means with the same superscript(s) are not significantly different; *Significant difference at P<0.05, **Significant difference at P<0.01, ^{ns} non-significant difference; [1]Maize silage (MS), Sweet sorghum silage (SS), Sweet sorghum silage treated with urea (SSU), Sweet sorghum silage treated with urea and molasses (SSUM), Sweet sorghum bagasse silage (BS), Sweet sorghum bagasse silage treated with urea (BSU), Sweet sorghum bagasse silage treated with urea and molasses (BSUM); [2]Standard error of the means for n=21 measurements; [3]Orthogonal comparison of MS *vs.* SS and BS; [4]Orthogonal comparison of SS *vs.* BS; [5]Orthogonal comparison of SSU and BSU *vs.* SS and BS *i.e.* effect of urea; [6]Orthogonal comparison of SSUM and BSUM *vs.* SSU and BSU *i.e.* effect of molasses; [7]Orthogonal comparison of SSUM and BSUM *vs.* SS and BS *i.e.* effect of urea plus molasses

Table 5: Protein fractions (g/kg of crude protein) of silages from maize, sorghum and sorghum bagasse with or without additives based on CNCPS method after 90 days of ensiling

	Silages[1]								Orthogonal contrasts				
Protein fractions[2]	MS	SS	SSU	SSUM	BS	BSU	BSUM	SEM[3]	MS *vs.* SS & BS[4]	SS *vs.* BS[5]	Urea[6]	Molasses[7]	Urea + Molasses[8]
A	616^{bc}	498^{d}	603^{c}	643^{abc}	460^{d}	702^{a}	691^{ab}	17.4	**	ns	**	ns	**
B_1	79	55	83	52	114	65	64	14.4	ns	*	ns	ns	ns
B_2	133^{ab}	176^{a}	103^{ab}	114^{ab}	140^{ab}	82^{b}	96^{ab}	18.6	ns	ns	**	ns	*
B_3	48	68	66	40	51	17	23	12.3	ns	ns	ns	ns	*
C	124^{c}	202^{ab}	145^{c}	152^{bc}	236^{a}	134^{c}	125^{c}	11.3	**	ns	**	ns	**

$^{a–c}$Within each row, means with the same superscript(s) are not significantly different; *Significant difference at P<0.05, **Significant difference at P<0.01, ^{ns} non-significant difference; [1]Maize silage (MS), Sweet sorghum silage (SS), Sweet sorghum silage treated with urea (SSU), Sweet sorghum silage treated with urea and molasses (SSUM), Sweet sorghum bagasse silage (BS), Sweet sorghum bagasse silage treated with urea (BSU), Sweet sorghum bagasse silage treated with urea and molasses (BSUM); [2]A: Non protein nitrogen (NPN), B_1: buffer soluble protein, B_2: protein with medium degradation rate, B_3: protein with slow degradation rate, C: protein insoluble in acid detergent; [3]Standard error of the means for n=21 measurements; [4]Orthogonal comparison of MS *vs.* SS and BS; [5]Orthogonal comparison of SS *vs.* BS; [6]Orthogonal comparison of SSU and BSU *vs.* SS and BS *i.e.* effect of urea; [7]Orthogonal comparison of SSUM and BSUM *vs.* SSU and BSU *i.e.* effect of molasses; [8]Orthogonal comparison of SSUM and BSUM *vs.* SS and BS *i.e.* effect of urea plus molasses

bagasse in the current experiment and the reported values (i.e. more CP and less NDF) can be attributed to either lower juice extraction or lower maturity stage of sweet sorghum in the current experiment.

Silage pH is an important factor in the long-term stability of ensiled plant material. A pH value below 4.0 is considered satisfactory for long-term storage of ensiled material (Jaster, 1995) as observed for all the silages in the current experiment. Results of the present study and other reports (Bolsen et al., 1985; Hinds et al., 1992) showed that urea increases silage pH and concentrations of NH_3-N and CP. Ensiling forages with urea increased concentrations of amino acids such as alanine, aspartic acid, glutamic acid, valine and isoleucine (Lessard et al., 1978). Soluble proteins could not be utilised optimally in the absence of adequate WSC, therefore molasses, a source of WSC, is often used along with urea to help preventing silage instability (Jaurena and Pichard, 2001). Many studies reported decreasing pH with the addition of molasses (Aminah et al., 2001; Baytok et al., 2005) while simultaneous addition of urea and molasses resulted in higher pH values (Keskin et al., 2005; Balakhial et al., 2008). This effect can be partly explained by the buffering capacity of silages which increases with the addition of urea (Berger et al., 1994). However, Guney et al. (2007) reported that the addition of 10 g/kg urea or 10 g/kg urea plus 50 g/kg molasses to the silage had no significant effect on pH.

Increased NH_3-N in the silages containing urea could be as a result of increased degrading activities of bacteria. Balakhial et al. (2008) observed that supplementing forages such as canola with urea can decrease silage quality by increasing pH value and NH_3-N concentration. Ammonia-N(NH_3-N) also can arise from other sources, such as the reduction of nitrates and nitrites, the action of lactic acid bacteria (Bergen et al., 1991), which are capable of amino acid

fermentation (Brady, 1960). Silage is considered excellent when the NH_3-N/N is below 7 g/100 g, and considered good when the NH_3-N/N is between 7 and 10 g/100 g (Romero, 2004). In our experiment, the NH_3-N/N ranged from 11.3 to 18.8 g/100 g with the greatest numbers for silages having urea plus molasses which indicates that the NH_3-N/N is greater than good silage. Nevertheless, the silages had good visual appearance, odour and colour, low final pH (*i.e.* 3.7–3.9), and absence of butyric and propionic acids indicating good fermentation (McDonald et al., 1991).

Bolsen et al. (1985) and Singh et al. (1996) reported that the addition of urea to sorghum silage increased the concentration of acetic acid whereas Hinds et al. (1992) reported no effect. Keskin et al. (2005) reported that the addition of 5 g/kg urea to sorghum silage had no effect on propionic acid concentration and 5 g/kg urea or 5 g/kg urea plus 40 g/kg molasses increased the butyric acid concentration. It is well known that the addition of molasses to silage increases lactic acid concentration (Bolsen et al., 1985; Hinds et al., 1992; Bolsen et al., 1996) and resultsin lower pH and lower NH_3-Nconcentration (Ojeda and Montejo, 2001). However, in the present study, the addition of molasses had no effect on lactate concentration. Decreasing aNDF and ADF concentrations of silages due to the addition of molasses may be as a result of lower fibre concentrations in the molasses (Bingol and Baytok, 2003).

Increased IVD_{DM} due to adding molasses to silages is consistent with Seoane et al. (1992) and Petit and Veira (1994) reporting that the addition of molasses to the silage increased digestibility due to increasing cell wall hydrolysis. These results are contrary to Keskin et al. (2005) who reported that the addition of urea or urea plus molasses to sorghum silages decreased the IVD_{DM} compared with the control. They attributed this decrease to increased organic matter (soluble carbohydrates) losses in the urea and molasses-containing silages. Di Marcoa et al. (2009) reported that the IVD_{DM} value at 24 h of incubation was the only indirect methodology that matched the corresponding *in vivo* data in all silages. High NDF digestibility of the silage is expected to be associated with increased feed intake and milk production (Oba and Allen, 2005). Each one percentage increase in estimated *in vitro* NDF degradability of maize silage based diet would increase the DM intake and milk production by 170g/d and 250g/d, respectively (Allen, 2000). Also there are positive effects of adding 6 g/kg DM urea to the lactating dairy cow feeding maize silage based diet and microbial protein synthesis was maximized in these animals (Boucher et al., 2007).

In the CNCPS, NPN is assumed to be converted rapidly to ammonia and does not contribute to the ruminal peptide pool, which is derived from the degraded true protein fractions. NPN is determined as the nitrogen passing into the filtrate after precipitation with a protein specific reagent (Licitra et al., 1996). When trichloroacetic acid (TCA) is used as a protein precipitant, peptides of less than ten amino acids units are not precipitated. Therefore, they are allocated to the NPN fraction. Because peptides and amino acids can stimulate microbial growth greater than ammonia (Russell et al., 1992), these solubilized peptides contribute to microbial growth, and allocating them to the NPN pool, results in the underestimation of microbial growth and MP allowable milk (Aquino et al., 2003).

When forages are ensiled, bacteria ferment the forage and breaks forage protein down into smaller fractions, which are more degradable by rumen bacteria. This process called proteolysis. Some researchers (Messman et al., 1994) estimated that only 9% of forage macro-protein molecules remain after fermentation. The decreases in the proportions of B_1, B_2 and B_3 fractions in the silages containing urea or urea plus molasses can be derived from the proportional effect of urea addition, which appears in the form of NPN (fraction A). The content of crude protein A fraction increased ($P<0.01$) from fresh to ensiled forage (mean 397.3 *vs.* 524.66 g/kg of CP, respectively), especially in maize silages. As a result, the content of true protein ($B_1+B_2+B_3$) decreased ($P<0.05$) similar to data reported for red clover by Krawutschke et al. (2011), they showed that the most important source of variation for all crude protein fractions was generally the ensiling stage, except for fraction C.

In current study, ensiling increased the B_1 fraction in maize and sweet sorghum bagasse ($P<0.05$) while adding urea or molasses or both of them simultaneously had no effect on B_1 fraction which is soluble in buffer and is mostly available to ruminal microorganisms (Krishnamoorthy et al., 1982). The B_2 fraction has a slower degradation rate than B_1 fraction and some of it escapes to the lower gut. Our results demonstrated that ensiling had most decreasing impact ($P<0.01$) on the B_3 fraction which has an even slower ruminal degradation rate than other B fractions, especially in bagasse silages (233 *vs.* 51 g/kg CP). The C fraction which considered unavailable in gastrointestinal tract (Krishnamoorthy et al., 1982), showed a reduction when urea plus molasses was added to the silages.

Conclusions

Higher DM and NFC concentrations in sweet sorghum plantand sweet sorghum bagasse compared with maize plant and similarity in pH and IVD_{DM} between MS, SS and BS silages indicated that sweet sorghum plant and sweet sorghum bagasse can produce silages with good nutritional value in arid environment

of central Iran. Also, similar pH values between MS, SS and BS silages but lower NH_3-N, lactate, acetate and ethanol in SS and BS silages compared with MS, indicated better fermentation pattern and lower DM losses in SS and BS silages compared with MS. Simultaneous addition of urea and molasses improves the nutritional quality of sweet sorghum and sweet sorghum bagasse silages by increasing CP, WSC, NFC and IVD_{DM} but reducing ethanol, aNDF and protein C fraction.

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