www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Influence of ensiling time on chemical composition, fermentation characteristics, gas production and protein fractions of sweet sorghum silage

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Abstract

The effect of ensiling duration on fermentation characteristics, chemical composition, gas production parameters and protein fractions of sweet sorghum silage was studied. Triplicate samples of sweet sorghum were ensiled for 0, 30, 60, 90, and 120 days in laboratory silos. Orthogonal contrasts were used to test linear, quadratic and cubic effects of ensiling time. The results of chemical analysis showed that, ensiling sweet sorghum increased dry matter (DM), soluble crude protein (SCP), ash and acid detergent lignin (ADL) concentrations compared to fresh sweet sorghum (P<0.01), however, net energy for lactation (NEl) and organic matter digestibility (OMD) decreased (P<0.01) compared to fresh material. The concentrations of ammonia nitrogen, lactic acid, acetic acid, ethanol and the amount of effluent increased significantly (P<0.01) with advancing ensiling period. The concentration of lactic acid was higher than other fermentation acids. DM and neutral detergent fibre (aNDF) concentrations decreased (P<0.01), while non-fibre carbohydrates increased linearly (P<0.01) with advancing ensiling process. The greatest amount of water soluble carbohydrate (WSC) loss occurred within the first 30 day of ensiling (from 166 to 96.7 g/kg DM) and this trend continued quadratically (P<0.01) with advancing ensiling time. Silages had lower (P<0.001) CP concentration compared with fresh forages (54.90 versus 61.80 g/kg DM) while time of ensiling did not affect (P<0.05) the CP concentration. Net energy for lactation (NEl) and organic matter digestibility (OMD) estimated from gas production technique were lower (P<0.01) for silages compared to fresh material and they decreased linearly (P<0.05) by advancing ensiling time. The results showed that, increasing ensiling time from 30 to 120 days decreased DM, WSC, aNDF and OMD concentrations and NEL content of sweet sorghum.

Keywords: Chemical composition; ensiling time; gas production; protein fractions; sweet sorghum

To cite this article: Zafari Naeini S, N Khodambashi Emami, E Rowghani, A Bayat, 2014. Influence of ensiling time on chemical composition, fermentation characteristics, gas production and protein fractions of sweet sorghum silage. Res. Opin. Anim. Vet. Sci., 4(6): 286-293.

Introduction

Traditionally, ensiling was used to preserve forage material for feeding animals after the cropping seasons. Recently, this method has a potential to store the large quantities of biomass feed stocks to produce energy through lignocellulosic ethanol production (Sipos et al., 2009). Similarly, there is a growing interest in sweet sorghum (*Sorghum bicolor*) in recent years because it has the potential to be used as bioenergy crop for lignocellulosic ethanol production. This crop is resistance to drought, saline and disease. In addition, it requires low fertilizer and it is an attractive forage crop for many tropical and subtropical areas including Iran. The high sugar content in sweet sorghum also makes to ensiled for dry season feeding. Therefore, there is an growing interest to study the possibility and convenience of partial or even total substitution of corn silage with sweet sorghum (Colombo et al., 2007). On completion of fermentation, pH, sugar and organic acid

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content should reasonably remain stable. Hydrolysis of hemicellulose due to the acidic condition is common during fermentation (McDonald et al., 1991). Fermentation by lactic acid bacteria is usually completes within three weeks (Jaster, 1995). However, Ward and de Ondarza (2008) suggested that, corn silage requires at least four months for a full fermentation process. Kleinschmit and Kung (2006) reported that a satisfactory fermentation of corn silage in mini silos requires 361 days of ensiling. In their investigation, the major increase in acetic acid in untreated corn silage occurred between 282 and 361 days, this evidence suggesting that the change in concentration in acetic acid was most likely due to *Lactobacillus buchneri.* This organism is relatively acid tolerant and can survive for long periods of time in fermented silage (Schmidt et al., 2009). Production of butyric acid by clostridia causes an increase in pH and significant losses in dry matter and energy content that can reach 50% and 20%, respectively (Bolsen, 1995). Studies have shown that aerobic microbial fermentation and the lactic acid bacteria (LAB) activities on plant cell respiration completes within the first month of ensiling (Gary, 1992). Caswell et al. (1983) ensiled sweet sorghum with moisture concentration of 740 g/kg for a period of 31 days and noted that WSC concentration was reduced by 57.5%. Similarly, Linden et al. (1987) ensiled compressed sweet sorghum containing moisture concentration of 660 g/kg and after 155 days of ensiling, 65% of the initial fermentable carbohydrate concentration was preserved. Stokes and Chen (1994) reported, after 56 days of fermentation, ADF, cellulose and CP in corn silage increased and NDF, hemicellulose, and WSC became lower than the original forage. Yahaya et al. (2002) showed that prolonging ensilaging time of high moisture orchard grass would result to excessive loss of DM, WSC, hemicellulose and cellulose in the silages. Calabrò et al. (2007) observed that the ensiling caused a reduction in NE_l, OMD, but an increase in structural carbohydrates contents in sorghum. Calabrò et al. *(*2007) showed that loss of soluble fractions during ensiling was higher for corn than sorghum. Hoffman et al. (2011) investigated the effect of ensiling time on starch-protein matrix in high-moisture corn and they reported that the NH_3-N and buffer soluble CP (SCP) concentration increased from 0 to 240 days. It was also been reported that rumen degradability of corn silage (Newbold et al., 2006) and high-moisture corn (Benton et al., 2005) increased with ensiling time. In addition, Philipp et al. (2007) noted reduction in WSC concentration for different varieties of sorghum over 21 days of incubation period.

Despite the importance of sweet sorghum as a bio-energy and forage sources crop, little research has been done on its utilization as an alternative feed resource during critical feed shortage especially in the dry season. Similarly, measurement of undesirable breakdown of nutrients such as WSC and CP during ensiling, which depends on many factors, is not fully understood for sweet sorghum. Furthermore, there is scanty information on the effect of ensiling time on the degradation of structural carbohydrates and alteration of *in vitro* digenstability (IVD) or gas production (GP) parameters and nutrients composition and lost during ensiling in sweet sorghum. The objective of this experiment was, therefore, to determine the effects of ensiling time on the chemical composition, nutritional characteristics and nutrients lost during ensiling in sweet sorghum forage after 30, 60, 90 and 120 days of ensiling in mini-silos.

Materials and Methods

Study location and crop management

The experiment was carried out at Isfahan University Research Station (31º, 31'N, 5º, 51' E, altitude 1550 m). Sweet sorghum was planted on 25 May, 2012 and harvested after about 120 days with a mean DM concentration of 295 g/kg fresh weight. Potassium sulfate (100 kg/ha) and ammonium phosphate (300 kg/ha) were used in the beginning of the cultivation. After 30 days of growing, 100 kg/ha urea were added to the field. Whole plant including stems, seeds and leaves were collected and chopped to about 2-3 cm in length using a mechanical forage cutter and divided into three equal parts by weight serving as experimental replicates.

Ensiling procedure

Forage pieces were ensiled in PVC containers with 4.0±0.2 kg capacity (cylindrical shape with 50 cm height × 16 cm diameter). Ensiling was done about 20 hours after harvesting so a slight wilting happened from harvest to ensiling time. A tap and hose were attached to the bottom of the silos to drain the effluent. After filling the silos with plant material, they were pressed using a pressing apparatus to expel the air completely. The silos were made air-tight by closing the lid tightly and the lids were lubricated with oil to seal effectively. The laboratory silos (three replicates for each treatment) were placed in a dark room at the average temperature of 18°C until their opening at 30, 60, 90 and 120 days after the storage.

Sampling and chemical analysis
Fresh and ensiled forage

The silages were evaluated after 30, 60, 90 and 120 days of ensiling. Before evaluation, from both sides of silos, about 5 cm of materials were discarded to ensure uniformity of samples. Then, the remaining material was mixed thoroughly. Approximately 2 kg of ensiled material was transferred into a vacuum plastic bag and preserved at -20°C for further experiment. Fresh forage and samples ensiled for 120 days were likewise frozen for 21- d to ensure protocol continuity (Hoffman et al., 2011).

Preparing silage extract

About 30-g sampled fresh silages was mixed with 270 ml distilled water and blended using a kitchen blender for 50 to 60 seconds. The extract was then filtered using four layers of cheesecloth. The pH was determined using a digital pH meter (Metrohm 744, Switzerland). Moreover, the extract was stored at -20°C for analyzing lactate, acetate, butyrate, propionate, ethanol and NH_3-N.

Chemical analysis

After being frozen for 21 days, all samples were allowed to thaw and were air dried for 48 hours in a forced-air oven at 55°C. Fine particles of silages were collected after sieving through a 1mm siever and were used to determine chemical composition. Effluent was estimated by volume gain (ml) of exhaust calibrated-collector tube connected via vessel to minisilos. The ether extract (EE), CP and ash were measured according to AOAC (1999) while 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).

Acetate, butyrate, propionate and ethanol were measured by gas chromatography (Crompak, Model CP 9002, The Netherlands) as described by Playne (1985). The lactic acid was estimated by high-performance liquid chromatography (HPLC) method developed by Megias et al. (1993). Ammonia nitrogen was measured using Kjeldahl method (Kjeltec Auto 1030 Analyzer, Sweden) in 50 ml of fresh silage extracts (without digestion) filtered through Whatman filter paper #1 (Filya, 2003). aNDF (using heat-resistant alpha-amylase and corrected for ash) and ADF were measured according to Van Soest et al. (1991) and ADL was measured by hydrolysis method using 72% sulfuric acid (Van Soest and Wine, 1968). The non fibre carbohydrates (NFC) were calculated using the following formula (Ishler and Varga, 2001):

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

Determination of the protein fractions

Borate-phosphate buffer (pH 6.7-6.8) and sodium azide 10% solution (freshly prepared) were used to measure SCP as described by Licitra et al. (1996). The NDIP and acid-detergent insoluble protein (ADIP or C fraction of CNCPS) were estimated according to Van Soest (1973).

Determination of in vitro digestibility

In order to determine *in vitro* digestibility of DM (IVD), 0.5 g of dried fine silage sample of smaller than 1mm was transferred into heat sealed F57 filter bags of Ankom and were incubated along with four empty bags. The buffer solutions A and B were prepared according to Ankom Daisy^{II} Incubators instruction (Ankom Technology, Macedon, NY, USA). Equal volume of the rumen fluid was obtained from 3 non-lactating Holstein cows (748.0±10 kg) consuming a total mixed ration about 4 hours after morning feeding which was mixed. A maintenance ration (AFRC, 1992) was fed in equal portions twice a day (07:00 and 19:00) consisting of 500 g/kg silage (1:1 maize silage: sweet sorghum silage), 350 g/kg chopped alfalfa and 150 g/kg concentrate. 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. The fluid was then filtered through four layers of cheesecloth. Each Ankom jar contained 400 ml filtered rumen fluid, 20 bags (four subsample for each sample), 266 ml B solution (15 g Na_2CO_3, 1 g $Na_2S.9H_2O$ per liter), 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 liter) 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} Fiber Analyzer, aNDF was determined based on the ANKOM protocol. The bags were dried at 60°C for 48 h and IVD was calculated.

Gas production

The *in vitro* gas accumulation was measured as described by Weimer et al. (2005). Approximately 200 mg of each sample was weighed into the graduated glass syringes of 100 ml. Three vials were placed as control (containing 30 ml mixture of rumen fluid and artificial saliva and no sample) in the beginning, middle and end of vial rows. Solution of micro mineral (13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$, 8 g $FeCl_3.6H_2O$ per 100 ml solution), rumen buffer (4 g NH_4HCO_3, 35 g $NaHCO_3$ per 1 litre of solution), macro mineral (5.7 g Na_2HPO_4, 6.2 g KH_2PO_4, 0.6 g $MgSO_4.7H_2O$ per 1 litre of solution), resazurine (1 g per 1 litre) and regenerative (4 ml NaOH 1 N, 625 mg $Na_2S.9H_2O$ and 95 ml distilled water) were prepared. The rumen fluid was collected and filtered from three ruminally fistulated non-lactating Holstein cows and was used for estimating *in vitro* true DM digestibility.

All procedures of handling rumen fluid were under continuous flow of CO_2. To vials, 10 ml rumen fluid and 20 ml buffer solution were added. Mixture was placed in a shaking water bath at 39.0±0.5°C for 30 min after the start of incubation. Rubber stopper was sealed with a light coating of petrolatum and vials were capped with butyl rubber stoppers, sealed with aluminium crimps. Gas pressures were measured with a digital pressure gauge (UniMano 1000, NeTech, USA) and the gas production (GP) was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours of incubation. The amount of GP was corrected for

blanks and gas production was fitted with the following model (Ørskov and McDonald, 1979):

$Y=b\ (1-e^{-ct})$. Where, *b* is the GP from the digestible fraction (ml), *c* is the GP rate constant (/h), *t* is incubation time (h), Y is gas production at time t.

ME, OMD and NE_l of samples using GP at 24 hours were estimated using equations described by Close and Menke (1986), Menke et al. (1979) and Menke and Steingass (1988), respectively:

ME (MJ/kg DM) =1.06 + (0.157×GP_{24}) + (0.0084×CP) + (0.022×EE) + (0.0081×CA)

OMD (g/kg DM) =148.8 + (8.89×GP_{24}) + (0.45×CP) + (0.0651×CA)

NE_l (MJ/kg DM) = 0.54+ (0.0959×GP_{24}) + (0.0038×CP) + (0.001733×EE)

Where, GP is gas production (ml/200 mg DM), GP_{24} is net gas production (ml/200 mg DM) at 24 h of incubation, CP is crude protein (g/kg DM), EE is ether extract (g/kg DM), CA is crude ash (g/kg DM).

Statistical analysis

Data were analyzed using the GLM of Statistical Analysis System (SAS, 2003). Orthogonal contrasts were used to test linear, quadratic and cubic effects of ensiling time. The statistical significance level was considered as P<0.05.

Results

Ensiled material had significantly higher DM, SCP, ash and ADL concentrations than original forage (Table 1). On the other hand, fresh material contains comparatively higher CP, NDIP, ADIP, WSC, ADF, IVD (Table 1) and OMD concentration and NE_l content (Table 4) than silage (P<0.01).

Table 1: Chemical composition of sweet sorghum forage and silage

Parameter[1]	Sweet sorghum		SEM[2]	P value
	Forage	Silage		
g/kg DM unless otherwise stated				
DM (g/kg fresh)	295[b]	346[a]	8.1	< 0.001
CP	61.8[a]	54.2[b]	0.82	< 0.001
SCP (g/kg CP)	460[b]	572[a]	7.4	< 0.001
NDIP (g/kg CP)	391[a]	256[b]	6.8	< 0.001
ADIP (g/kg CP)	219[a]	199[b]	5.6	< 0.01
WSC	166[a]	73.4[b]	8.48	< 0.001
Ash	57.8[b]	62.7[a]	1.60	< 0.05
aNDF	514	516	12.2	0.90
ADF	302[a]	249[b]	6.1	< 0.001
ADL	47.7[b]	69.1[a]	4.97	< 0.01
NFC	342	335	11.1	0.58
IVD	699[a]	686[b]	3.3	< 0.01

[1]DM, dry matter; CP, crude protein; SCP, buffer soluble CP; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; WSC, water soluble carbohydrates; aNDF, neutral detergent fibre corrected for ash; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC, non-fibre carbohydrates; IVD, in vitro digestibility; [2]Standard error of the means n=15

A quadratic decreasing (P<0.01) trend in silage pH with increasing ensiling time was observed (from 3.88 to 3.74 for 30 and 120 days of ensiling, respectively). The concentrations of NH_3-N, lactic acid, acetic acid and ethanol increased (P<0.01) as the ensiling time advanced from 30 to 120 days (Table 2). But, propionic and butyric acids were not detected in sweet sorghum silages.

A linear decrease (P<0.01) of DM, WSC and aNDF with prolonged ensiling time of sweet sorghum was observed (Table 3). A significantly highest (quadratic trend) buffer SCP concentration in 90 days silage was observed (Table 3). The NFC concentration of fresh sweet sorghum decreased non-significantly during the first 30 days of ensiling (342 versus 308 g/kg DM), but increased to 354 g/kg DM by days 120 of ensiling. On contrary, prolonging the ensiling time decreased linearly (P<0.05) GP_{24} and NE_l content (Table 5).

Discussion

Silage pH is an important parameter in the long term stability of ensiled forages. For instance, forage with low buffering capacity allows a pH drop rapidly even when acid production is small (Alli et al. 1983). A pH below 4.0 is considered satisfactory for long term storage of ensiled material (Jaster, 1995). In this study, propionic or butyric acids were not detected. This may be due to acidic environment unsuitable for the entobacteria and clostridia deleterious activity, but promotes chemical hydrolysis of hemicellulose (McDonald et al. 1991). The decrease in silage pH in this experiment is similar to those reported in previous studies ensiling sweet sorghum by Philipp et al. (2007).

With advancing ensiling time lactic and acetic acids increased. High acetic acid concentration may reflected fermentation of pentose sugars released from hemicellulose fraction to equal proportions of lactic and acetic acids by hetero-fermentative LAB **(**McDonald et al. 1991). Schmidt et al. (2009) reported that the population of LAB in alfalfa silage (without additives) peaked between 5 and 45 days (>9 log CFU/g) of ensiling and increased further after 180 days of ensilage. In present study, increasing fermentation end products (lactate, acetate and ethanol) after 3 months of ensiling suggest that microbial activity persists even at low pH condition as claimed by Kung and Der Bedrosian (2010). *Lactobacillus buchneri* appears to be active for longer duration in corn silage and probably this contributes to the present findings. Under anaerobic conditions and low pH, this organism is able to convert lactic acid to acetic acid, ethanol and 1, 2 propanediol (Oude-Elferink et al., 2001). Silages with high ethanol concentration are an indicative of slow decline in pH of ensiled material and having higher final pH values. Driehuis and Wikselaar (2000) reported that grass silages with 48 to 63 g ethanol/kg DM increase the silage pH more than 5.3.

Table 2: Fermentation characteristics of sweet sorghum silage at different ensiling times

Parameter	Ensiling day				SEM[1]	P value[2]		
	30	60	90	120		L	Q	C
pH	3.88	3.76	3.76	3.74	0.010	< 0.001	< 0.01	< 0.05
g/kg DM otherwise stated								
NH_3-N	0.50	0.54	0.60	0.70	0.011	< 0.001	< 0.05	0.71
NH_3-N (g/kg total N)	56.9	62.1	69.4	81.7	1.34	< 0.001	< 0.05	0.63
Lactic acid	21.0	24.1	25.6	25.9	0.17	< 0.001	< 0.001	0.60
Acetic acid	8.26	10.04	10.74	11.44	0.643	< 0.01	0.42	0.71
Lactate/acetate	2.68	2.40	2.38	2.17	0.242	0.19	0.87	0.69
Ethanol	1.51	1.61	1.80	1.96	0.032	< 0.001	0.44	0.41
Effluent (ml/kg fresh)	7.89	10.06	10.74	10.87	0.462	< 0.01	0.06	0.66

[1]Standard error of the means n=12; [2]L, linear effect of time; Q, quadratic effect of time; C, cubic effect of time

Table 3: Chemical composition of sweet sorghum silage at different ensiling times

Parameter[1]	Ensiling day				SEM[2]	P value[3]		
	30	60	90	120		L	Q	C
g/kg DM otherwise stated								
DM (g/kg DM as fed)	359	355	341	328	4.7	< 0.01	0.36	0.62
CP	54.9	54.3	54.0	53.5	0.85	0.25	0.95	0.88
SCP (g/kg CP)	556	578	582	573	6.06	0.09	< 0.05	0.87
NDIP (g/kg CP)	267	253	252	251	6.9	0.15	0.38	0.67
ADIP (g/kg CP)	201	200	196	196	6.8	0.53	0.91	0.81
WSC	96.7	69.2	66.5	61.2	2.51	< 0.001	< 0.01	< 0.05
Ash	61.0	62.8	63.5	63.6	1.78	0.30	0.65	0.93
aNDF	544	517	508	495	8.9	< 0.01	0.46	0.59
ADF	244	249	253	252	6.83	0.37	0.74	0.93
ADL	67.8	64.4	72.2	72.3	4.65	0.33	0.72	0.39
NFC	308	338	340	354	7.6	< 0.01	0.30	0.28
IVD	689	688	687	680	4.3	0.09	0.37	0.77

[1]DM, dry matter; CP, crude protein; SCP, buffer soluble CP; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; WSC, water soluble carbohydrates; aNDF, neutral detergent fibre corrected for ash; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC, non-fibre carbohydrates; IVD, *in vitro* digestibility; [2]Standard error of means, n=12; [3]L, linear effect of time; Q, quadratic effect of time; C, cubic effect of time

Table 4: The gas production parameters of fresh sweet sorghum forage and silage

GP Parameter[1]	Sweet sorghum		SEM[2]	P value
	forage	silage		
GP_{24}	192[a]	179[b]	2.70	< 0.01
b	360	345	10.63	0.25
c	0.049	0.044	0.0033	0.17
OMD	522[a]	496[b]	4.9	< 0.01
ME	8.59	8.37	0.129	0.16
NE_l	4.50[a]	4.24[b]	0.064	< 0.01

[1]GP_{24}, gas production in 24 hours (ml/g DM); "*b*", is the GP from degradable fraction (ml); "*c*", constant rate of gas production (/h); OMD, organic matter digestibility (g/kg DM); ME, metabolizable energy (MJ/kg DM); NE_l, net energy for lactation (MJ/kg DM); [2]Standard error of means n=15

At ensiling, fresh sweet sorghum forage there was no appreciable quantity of NH_3-N. However, the NH_3-N concentration increased steadily from 30 to 120 days for sweet sorghum silages. NH_3-N is a product of bacterial deamination of amino acids rather than a product of acid hydrolysis of silage VFA (Ohshima & McDonald 1978). Filya (2003) reported increasing amount of NH_3-N in corn and sorghum materials through 90 days of ensiling. Kleinschmit and Kung (2006) reported a steady increase in NH_3-N in corn silage through 361 d of ensiling without reaching a plateau. Silage is considered as an excellent and good when the NH_3-N/TN is below 7 g/100 gTN while and 7-10 g/100 gTN, respectively (Romero, 2004). As per the criteria, silage ensiled for 90 and 120 days in the present study may be categorized as an excellent and good quality, respectively.

NDF concentration of silage decreased between 30 to 120 days of ensiling which might be attributed to degradation of cell wall by activity of bacterial enzymes (cellulase and hemicellulase) and production of organic acids during fermentation (Yahaya et al., 2001). The NDF of sweet sorghum silages increased after first 30 days of ensiling, but decreased until day 120. Similarly, Henk and Linden (1992) reported that NDF content of sweet sorghum silage increased between day 4 and 7 of fermentation before continuing to decline. The reason of this change in NDF concentration is yet to be documented however, the high WSC content in sweet sorghum may have resulted to rapid decrease in the concentration of WSC in the first 30 day of ensiling (from 166 to 96.7 g/kg DM) which might increase the ratio of NDF to DM.

Table 5: The gas production parameters of sweet sorghum silage at different ensiling times

Parameter[1]	Ensiling day				SEM[2]	P value[3]		
	30	60	90	120		L	Q	C
GP_{24}	184	182	177	174	2.7	< 0.05	0.78	0.56
b	351	356	353	321	10.6	0.08	0.10	0.67
c	0.045	0.045	0.041	0.042	0.0033	0.38	0.85	0.61
OMD	504	501	491	486	5.4	< 0.05	0.80	0.61
ME	8.48	8.47	8.31	8.22	0.136	0.16	0.78	0.72
NE_l	4.32	4.30	4.19	4.13	0.059	< 0.05	0.80	0.62

[1]GP_{24}, gas production in 24 hours (ml/g DM); "*b*", is the GP from degradable fraction (ml); "*c*", constant rate of gas production (/h); OMD, organic matter digestibility (g/kg DM); ME, metabolizable energy (MJ/kg DM); NE_l, net energy for lactation (MJ/kg DM); [2]Standard error of means, n=12; [3]L, linear effect of time; Q, quadratic effect of time; C, cubic effect of time

WSC concentration in silages decreased dramatically by 69.3 g/kg during the first 30 day of fermentation and continued to gradually fall in second 30 day (27.5 g/kg) but after day 60 the decrease was negligible. These data shows the rapid microbial utilization of sugars in early stages of fermentation at low pH. This is confirmed by the continued small reduction in silage pH with increase in ethanol production as mentioned by Stokes and Chen (1994). In addition, the DM content reduced as ensiling progressed. For example, 328 and 359 g/kg of DM were observed in 120 and 30 day sweet sorghum silage preparation. The decrease could be explained by a moderate fall in reducing sugars which is similar to Henk and Linden (1992) and Stokes and Chen (1994) findings for sweet sorghum and corn silage, respectively. This reduction was ascribed to the continued maintenance requirement of the microbial population in the sweet sorghum silage.

The cellulose content (data are not presented) was not affected (P>0.05; SEM 5.682) by ensiling time while hemicellulose decreased (P<0.05; SEM 13.63) about 57 g/kg of DM likewise. Morrison (1979) showed that the core lignin concentration of forage did not change and cellulose could decrease to 50 g/kg during ensiling after 150 day of storage. Yahaya et al. (2001) confirmed that considerable loss of the hemicellulose and pectin fractions occurred in alfalfa and orchard grass silage between fresh forage and ensiled materials.

Studies report that the respiratory process accounts greater loss of silage DM and reducing the quality of material(Bolsen, 1995) due to heating and conversion of sugars and organic acids to undesirable products such as NH_3, CO_2, and H_2O (Stokes and Chen, 1994). By contrast, our study observed increase in DM content in the first 30 day of ensiling (from 295 g/kg of fresh forage to 359 g/kg in day 30) and latter decreased linearly to 328 g/kg at day 120.

In our study, ensiling time had no significant effect on IVD of sweet sorghum silages. Similarly, Der Bedrosian et al. (2010) reported that time of ensiling did not affect the *in vitro* NDF digestibility of two corn silage hybrids between 45 and 315 day of ensiling. Furthermore, the length of storage (up to 180 day) had no effect on the digestibility of cell walls as evaluated by *in vitro* gas production (Cone et al., 2008). Increased *in vitro* digestion of starch in normal and brown mid-rib corn silage hybrids through 270 day of storage has been reported by Der Bedrosian et al. (2010). In contrast, increasing the time of ensiling had no affect on starch digestion during *in vitro* gas production system (Cone et al., 2008). Digestibility is highly influenced by fibre and sugar concentrations in the forage. For instance, the lower NDF: sugars ratio there would be the higher IVD (Rodrigues et al., 2001). Therefore, simultaneous decreases in NDF and sugar contents during the ensiling process resulted to insignificant effect on IVD in current experiment. This is not in agreed with the findings of Pedroso et al. (2005) and Siqueira (2005). They report that ensiling the high WSC content forages such as sugarcane causes a greater loss of WSC, but increases the fibre components and thus reduces IVD in silage.

Conclusion

This study showed that the duration of ensiling changed characteristics of sweet sorghum silage. Despite strong acidic condition, anaerobic activity process continued till the end of our experiment (120 days of ensiling). Therefore, there is no need for silage additives to maintain the silage quality for four months.

Acknowledgement

The authors are grateful to the management board of the Shamim Roshd Espadan Co. and Mr.Changizi for providing materials and monetary fund to undertake this study.

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