In situ Rumen Degradation Characteristics of Maize, Sorghum and Sorghum-Sudan Grass Hybrids Silages as Affected by Stage of Maturity

Research Article

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ABSTRACT

This research was conducted to investigate in situ degradation characteristics of maize, sorghum and sorghum-Sudan grass hybrids. Whole plant of maize (ITM-815, DK-711), sorghum (SS-506, FS-5) and sorghum × Sudan grass hybrids (P-988, Grazer N2) were grown under semi-arid conditions and harvested at different maturity stages (mid-flowering (MF), milk-line (ML) and hard-dough (HD)) and ensiled. Three replicate silage samples were incubated at 0, 12, 24, 36, 48, 72 and 96 h in three rumen fistulated Holstein heifers. Effects of species had a large impact on rumen degradation characteristics values (a, b, (a+b), c), effective dry matter degradability (EDMD) and metabolizable energy (ME) MJ/kg for maize (M), sorghum (S) and sorghum × Sudan grass hybrids (SSH) silages. Effective dry matter degradability (EDMD) of dry matter was found as 286.65, 259.37, 265.0 g/kg for species silages, respectively (P<0.0001). Acid detergent fiber (ADF) was found to be the best single predictor of effective dry matter degradability of sorghum × Sudan grass hybrids silages (P<0.05, R=0.448).

KEY WORDS harvesting stage, maize, metabolizable energy, rumen degradation characteristics, sorghum, sorghum × Sudan grass hybrids.

INTRODUCTION

Maize (M), sorghum (S) and sorghum × Sudan grass hybrids (SSH) whole plant silages continue to be a major forage and energy source in the dairy industry for most countries. Economic pressures during the last decade related to the high costs of grain and forage reduced profit margins have resulted in a renewed interest in forage quality of whole plant maize (Johnson et al. 1999). Besides, sorghum has a high productivity potential and a high content of non-fibrous carbohydrates, mainly starch, which are desirable attributes of dairy cattle forages. The effective use of genetic diversity around the world by plant breeding programs has led to rapid changes in the type of S grown (Rooney et al. 1986).

Phenological stage is one of the most important factors affecting nutritional quality of forage. Fariani et al. (1994) reported that nutritive value of forage depend on morphological and physiological changes. Advancing maturity of fodder crops, the cytoplasmic portion of cell reduces. The quality of soluble carbohydrates, lipids, proteins and soluble proteins decrease. Additionally, Khan et al. (2011) reported that dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents of maize, sorghum and millet silages increased with the advancement of fodder growth.

However, crude protein (CP), total digestible nutrients (TDN) and metabolizable energy (ME) contents of three silages decreased as the fodders advanced in age.
The water soluble carbohydrate (WSC) content, pH and NH₃-N of maize, sorghum and millet silages decreased as the fodder advanced stage. Hunt et al. (1989) and Resende et al. (2003) studied on the maturity effects of six maize hybrids for two years at two locations. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the whole plant decreased, as the maturity proceeded from early one-third milk-line (ML) to mid two-thirds maturity and did not change from mid to late (black layer) maturity.

Additionally, harvest of whole plant corn (WPC) at a mature stage may increase whole kernel passage and lower starch digestibility, resulting in a lower energy density. Neither stover nor starch digestibility was considered in most equations that predict the energy value of silage from WPC.

Gül et al. (2008) reported that dry matter (DM) digestibility of M cultivars was higher than S and SSH at 24 h rumen incubation. Besides, pH differences in silages were expected due to higher concentrations of water-soluble carbohydrates. pH values and lactate concentrations were indicative of adequate preservation. Optimal harvesting stage to increase yield and silage quality of maize (Rafiuddin et al. 2016), in literature, varied from harvesting stage (Fu et al. 2011), one-third milk line (Johnson et al. 2002), mid-bloom (Rafiuddin et al. 2016), late dough stage (Vecchiettini et al. 2003) two-thirds milk line stage (Fariani et al. 1994). When harvested sorghum at late-milk, late-dough and hard-grain stages of maturity, higher nutritive values were noticed at late milk stage silages (Sonon and Bolsen, 1996). The objective of current study was to examine the effect of varying maturity stages of three different species; maize (TTM-815, DK-711), sorghum (SS-506, FS-5) and SSH (P-988, Grazer N2) silages on chemical composition and ruminal degradation characteristics under the semi-arid climatic conditions.

**MATERIALS AND METHODS**

**Field trial and silage samples**

Field trials were conducted in semi-arid region in Diyarbakir. Cultivars were planted as the second season crops. Average annual rainfall, temperature and relative humidity of experimental area were 481.6 mm 15.8 °C and 53.8%, respectively. Average temperature can reach 30 °C in summer months. Whole plant of maize (TT-815, DK-711), sorghum (SS-506, FS-5) and SSH (P-988, Grazer N2) were harvested at different maturity stages of mid-flowering (MF), milk-line (ML) and hard-dough (HD) and silaged in GAP International Agricultural Research and Training Center, Diyarbakir, Turkey. After harvesting forage on three dates and at approximately 2 week intervals, plants were chopped about 1.5 cm length and ensiled in 10 L jars for 60 days (Ashbell et al. 1991). After ensiled period, the forages silages were opened and examined. Then, all silage samples were brought to Adana and started to be evaluated within the scope of in situ studies at the animal feeding laboratory of Institute.

**Chemical analysis**

pH were analysed by taking approximately 50 g of sample in duplicate and then diluted with 360 mL of distilled water in a blending jar. Samples were stirred for 3 min. After filtration pH was measured by using a glass electrode pH meter (Mettler Toledo S-220). Besides, 500 g silage sample was dried at 70 °C and ground with mill 1 mm screen for determination of DM and chemical analysis. Crude protein (CP) content of the forage was determined by using kjeldahl method using tecator block digestion and steam distillation (multiplying total N by 6.25) (AOAC, 1990). ADF and NDF content of silage samples were determined by using Van Soest (1994) as adapted ANKOM Fiber Analyzer (F220/220 Operator’s Manual, Ankom tech.) filter bag method. The washing losses (WL) were determined in the following procedure (Undersnder et al. 1993; Lai and Thu Huong, 1999). The samples (1 g) were put in bags (50×150 mm) made from nylon filter cloth with a pore size of 45 to 55 microns and then bags were washed at three consecutive cycles of 30 min each in the washing machine. Three liters per bag water was used in every cycle. After those bags were dried at 55 °C in an oven for 48 h and subsequently, washing loss of dry matter was determined.

**In situ incubations**

Silage samples were dried and then samples were prepared by grinding through laboratory hammer mill with a 2.5 mm screen. Samples (5 g) were placed in a nylon bags (bags made of polyester and 7.5 cm²/15.5 cm, 40 micron pore size). Rumen fistulated (10 cm diameter, Diamond Inc) of 4-yr-old 3 Holstein heifers and with an average body weight (BW) of 450±30 kg were used to evaluate forages. Nylon bags were put into the rumen for incubations of 0, 12, 24, 36, 48, 72 and 96 hours. Each of heifers was fed on alfalfa (70%) and grass (30%) forage based diet ad libitum as recommended by NRC (2001) twice a day at 08:30 and 14:00 h and given mineral-vitamin premix (one kilogram of premix contains the following: 400 g limestone, 100 g calcium perphosphate, 200 g salt, MgO 90 g, vit A 32000 IU, vit D 5000 IU, vit E 165 mg/kg, Fe, 1500 mg, Cu 685 mg, Zn 2500 mg, Mn 1500 mg, Se 80 mg, I 30 mg and Co 25 mg), salt and fresh water during the trial. Heifers were housed in individual pens and allowed to adapt to the experimental conditions during 3 weeks. All feed samples of silage were prepared and incubated as three replicates to the rumen of fistulated animals. After incubation, samples were
withdrawn from the rumen. Bags were washed in cold water. Solubilized DM at the beginning of incubation (time 0) disappearances were obtained by washing non-incubated bags of similar fashion and then bags were oven dried at 55 °C for 48 h. *In situ* dry matter degradability for each incubation period was calculated by the equation:

\[
\text{DM (g/kg)} = \frac{\text{initial weight-final weight}}{\text{initial weight}} \times 100
\]

Degradation (digestion) characteristics of DM were calculated using the equation (Ørskov and Mc Donald, 1979; Van Soest et al. 1991).

\[
D_{(DM)} = a + b (1-e^{-ct})
\]

Where:

- \(D\): disappearance rate of DM at time \(t\).
- \(a\): intercept representing the portion of DM solubilized at the beginning of incubation (time 0).
- \(b\): portion of DM that is slowly degraded in the rumen.
- \(c\): rate constant of disappearance of ‘\(b\’\).

The parameters \(a\), \(b\), \(c\) and effective dry matter degradability (EDMD) were calculated (Van Soest et al. 1991).

\[
\text{EDMD (1, 2, 3)} = a + b \times \left(\frac{c}{c+k}\right)
\]

Where:

- \(k\): ruminal outflow rate, being \(k_1 = 0.02/\text{h}\), \(k_2 = 0.05/\text{h}\), \(k_3 = 0.08/\text{h}\).

**RESULTS AND DISCUSSION**

**Washing loses**

Washing loses of dry matter of M, S and SSH were determined that there was significantly (\(P<0.0001\)) difference between M and the other species (S and SSH) as presented in Table 1. But, there were no difference among the cultivars (TTM-815, DK-711, SS-506, FS-5, P-988, Grazer N2). As seen in Table 1, advancing maturity stage of plants, WL values of dry matter increased from MF to HD. Washing loses (WL) of sorghum increased up to ML then decreased down to HD stage.

**Chemical analysis**

Difference of crude protein content of maize and sorghum were significant (\(P<0.0001\)) in comparison to SSH silage (Table 1). Additionally, although there were important differences between CP of S and SSH cultivars (\(P<0.05\)), no difference was found within CP of M cultivars. CP of M was significantly declined (\(P<0.0001\)), as maturity advanced from MF to HD stage. Although CP of S and SSH from MF to HD did not change throughout the maturity. NDF content of M, S and SSH was significantly differed (\(P<0.0001\)). Between cultivars were no difference observed. NDF content of M was declined from MF to HD stage as the maturity advanced. There was significant difference between MF to HD stage (\(P<0.0001\)) as illustrated in Table 2. Although the first NDF content of M was increased from MF to ML stages, then it was declined from ML to HD throughout the maturity. There was no difference of NDF content of S and SSH between the cutting stages. ADF content values of all species declined as the maturity advanced from MF to HD. ADF content was significantly (\(P<0.0001\)) different among the cutting stages (Table 2).
In situ Rumen Degradation

Among the species (M, S and SSH), there were no significant difference in terms of silage pH as seen in Table 3. But, pH was affected with harvesting stages of species. Therefore, pH significantly declined from MF to HD stage (P<0.001).

Rumen degradation characteristics
Rapidly soluble fraction (a), potentially degradable fraction (b) and total degradable fraction (a+b) were significantly (P<0.0001) between M and the other species silages. As well EDMD1, EDMD2 and EDMD3 of M were significantly (P<0.0001) different from those of S and SSH silages. Rate constant (c) was more rapid for M and S than those for SSH parameters (Table 4).

Highly significant differences were observed between M, S and SSH (P<0.0001). Besides, metabolizable energy content of SSH was higher than that of M and S species. There were strongly significant differences between SSH and M silages (P<0.0001).
Table 3. Effects of cutting stage and species on pH values of silages

<table>
<thead>
<tr>
<th>Cutting stage</th>
<th>N</th>
<th>SEM</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-flowering</td>
<td>4.98a</td>
<td>4.41b</td>
<td>4.31b</td>
</tr>
<tr>
<td>Milk line</td>
<td>54</td>
<td>0.131</td>
<td>&gt; 0.953</td>
</tr>
<tr>
<td>Hard dough</td>
<td>54</td>
<td>0.131</td>
<td>&gt; 0.953</td>
</tr>
</tbody>
</table>

Table 4. Effects of cutting stage and species on pH values of silages

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>SEM</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>18</td>
<td>6.391</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sorghum</td>
<td>18</td>
<td>9.038</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SSH: Sudan grass hybrids</td>
<td>18</td>
<td>9.038</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

** (P<0.001) and *** (P<0.0001).

SEM: standard error of the means.

Table 5. The effects of maturity stage and species on rumen degradation characteristics and metabolizable energy of silages

<table>
<thead>
<tr>
<th>Species</th>
<th>Cutting stage</th>
<th>ME (MJ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>MF</td>
<td>8.31 b</td>
</tr>
<tr>
<td></td>
<td>ML</td>
<td>7.29 a</td>
</tr>
<tr>
<td></td>
<td>HD</td>
<td>7.71 ab</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&lt; 0.086</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.38</td>
</tr>
<tr>
<td>Sorghum</td>
<td>MF</td>
<td>8.36</td>
</tr>
<tr>
<td></td>
<td>ML</td>
<td>7.93</td>
</tr>
<tr>
<td></td>
<td>HD</td>
<td>8.60</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&lt; 0.086</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.38</td>
</tr>
</tbody>
</table>

SSH: Sudan grass hybrids; EDMD: effective degradability and ME: metabolizable energy.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

** (P<0.001) and *** (P<0.0001).

SEM: standard error of the means.
However, differences were not significant when compared the cultivars M (TT-815, DK-711), S (SS-506, FS-5) and SSH (P-988, Grazer N2), in terms of ME and rumen degradation characteristics (Table 4). Rapidly soluble fraction (a) and EDM: for M increased with plant maturity stage advanced from MF to HD stage. Highly significant differences were observed between MF and HD (P<0.001), when the cutting stage of maturity advanced from MF to HD (Table 4). Soluble fraction of (a) was not affected by the cutting stage (P=0.086).

The ruminal degradation characteristics of species and cultivars silage are presented in Table 4 and Table 5. Rapidly soluble fraction (a) of M and SSH plants was affected with different harvesting stage (P<0.0001). There also were significant difference among species in terms of rapidly soluble fraction (a). Maize had highest value of rapidly soluble fraction (a) of M and SSH plants was affected with different harvesting stage (P<0.0001). There also were significant difference among species in terms of rapidly soluble fraction (a). Maize had highest value of rapidly soluble fraction (a) among the other species (M, S and SSH).

The greatest value of EDMD_2 of S was obtained at ML cutting stage among the other stages. It is apparent that there was linear and quadratic effect for harvesting stage; but no significant difference was found among the cutting stages for S (P=0.086). Parameter (a) for SSH gets increased with the harvesting stage advanced from MF to HD. Highly significant differences were found among the cutting stages of maturity of hybrids from MF to HD (P<0.001). Potential degradable fraction (b) was increased, when the harvesting stage advanced from MF to ML for M. Differences in harvesting stage between species and differences of cutting stage maturity on rumen degradation characteristics of M, S and SSH are shown in Table 4 and Table 5 respectively. Then, it decreased down to the HD stage. The value for ML cutting stage was higher than those for other harvesting stages. There were quadratic effects for the harvesting stages (P=0.057). However, differences among the cutting stages of maturity of S and SSH were not significant. The degradation rate constant (c) of DM was found greater at ML than MF and HD stages for M. The (c) fraction decreased, as the plant maturity stage advanced from MF to HD. There were significant differences between HD and the other stages (P<0.001). Although three stages of maturity have no effect on (c) fraction for SSH. Significant differences were observed between HD and the other stages (P<0.001). DM degradability constant (c) for S was decreased from first cutting stage to third harvesting stage (Table 5). Cutting stage of maturity of S had a significant (P<0.01) effect on constant (c). Calculated fraction for M, S and SSH increased with plant maturity stage advanced from MF to HD. There were significant differences among the different cutting stages of maturity for species varying in maturity from MF to HD (P<0.0001). Besides, fraction (a) and rate of degradation kinetics (c) decreased from MF towards to HD stage (Table 6).

**Prediction of rumen degradation characteristics and metabolizable energy**

Regression equations, describing the correlation between DM rumen degradation characteristics and chemical composition of M, S and SSH silages are presented in Table 7. ADF was found to be the best single predictor of parameter (a) of M silage. There was a negative correlation between ADF and parameter (a) (P<0.05, R^2=-0.39). Combination of ADF, NDF and CP predicted the (a) value was shown in Table 7. While negative correlation determined among the ADF, NDF and parameter (a), in contrast, CP and parameter (a) correlation was found as positive for M silages (P<0.05, R^2=0.507). ADF was found to be the best single predictor of parameter of rapidly soluble fraction (a) of SSH silages where there was a negative correlation between them (P<0.05, R=-0.446). In addition, there seems to be a negative correlation between rapidly soluble fraction and cellul wall (NDF and ADF) combination (P<0.05, R^2=-0.531). CP content was negatively affected by rapidly soluble fraction (a) of SSH silages (P<0.01). As seen in Table 7. ADF and NDF were found to be two important predictors of effective degradability (EDMD) of M. There was a negative correlation between EDMD and cell wall (ADF, NDF) content of M silage (P<0.05, R^2=-0.405). ADF also was found to be the best single predictor of effective dry matter degradability of SSH (P<0.05, R^2=0.448). There was a positive correlation between CP and ME (P<0.01, R^2=0.344). A regression with three predictors (NDF, ADF, CP) showed that there were positive and significant coefficient of correlation among them (R^2=0.494, P<0.05).

In current study, the findings of WL with increasing maturity was consistent with previous studies of Tabacco et al. (2004) and Khan et al. (2007). Who reported that water soluble carbohydrate (WSC) contents of M, S and millet increased with advancing maturity due to accumulation of starch. Maize contained more CP than S and SSH as Resende et al. (2003), Khan et al. (2007) and Podkowka and Podkowka (2011) reported. Crude protein concentration of M declined from MF to HD harvesting stage in similar with Bal et al. (1997), Coors et al. (1997), Johnson et al. (1999) and Khan et al. (2007). Besides, Crude protein contents of S and SSH did not change during the maturity stage. NDF concentration of M was lower than SSH in consistence with the finding similar figures of Khan et al. (2007). In addition, maize NDF content was increased by 1.44% from MF to ML and then decreased by 5.76% from ML to HD maturity stage.

Bal et al. (1997) reported that declining NDF content was related to the increase in the proportion of grain in whole plant corn at the harvesting stage. ADF containing M, S and SSH decreased as maturity progressed from MF to HD stage.
Similar trends for NDF and ADF have been reported by other researchers like Coors et al. (1997), Marco et al. (2002) and Johnson et al. (1999).

The linear decrease in pH values of maize, sorghum and SHH silages from MF to HD stage of maturity was in agreement with the findings of Rafiuddin et al. (2016). Khan et al. (2011) also reported that ensiled forage (maize, sorghum and millet) at initial stage of growth did not decrease pH quickly. This may be described by the presence of higher concentrations of rapidly soluble carbohydrates (Bal et al. 1997). pH value also was indicative of adequate preservation (Fisher and Burns, 1997).

But, pH results of this study were higher than findings of Podkowka and Podkowka (2011). Ruminal degradation characteristics results were determined for the SSH silages. Similar findings were reported by Resende et al. (2003).

Effective ruminal dry matter degradabilities (EDMD1, EDMD2 and EDMD3) of S and SSH silages were lower than M silage. Loveras (1990), Resende et al. (2003) and Pour et al. (2012) reported that EDMD of S was lower than M. These findings may suggest that the endosperm in S grain is more vitreous than that in maize. This fact could be explained, the peripheral endosperm region is extremely dense, hard and resistant to water penetration and digestion. Peripheral cells, with high protein content and resisting to both physical and enzymatic degradation, were possibly limiting the starch degradation in the rumen (Rooney and Pflugfelder, 1986; Theurer, 1986; Johnson et al. 1999).
Beck et al. (2007) reported that SSH trial was conducted at three harvesting stages and that in situ dry matter kinetics (a), (b), (c) and EDM decreased from the first harvesting stage to the third harvesting stage.

Current study results similars with the findings of Hatew et al. (2016) reported that increased maturity of whole-plant corn from 25 to 40% DM at harvest reduced in situ effective ruminal starch degradability by 13%.

The (c) fraction decreased, advancing maturity stage of species in agreement with the findings of Hatew et al. (2016) reported that the ruminal fractional rate of degradation of starch and NDF decreased linearly with increased maturity of whole-plant corn at harvest

ME values of species did not change at three cutting stages. Similar findings were reported by St. Pierre et al. (1987) that apparent DM and energy digestibility were not different in M silage varying in maturity from 21 to 46% DM.

Additionally, Buck et al. (1969) reported that the stage of maturity ranging from 22% to 34% DM (trial 1) and 32% to 40% (trial 2) did not alter digestible energy estimates

Generally, NDF was the best predictor parameter. NDF represents the total insoluble matrix fiber, and it is much more related to rumination and passage compared to other chemical components (Van Soest, 1994). As result of regression equations, CP was found to be the best single predictors of ME.

Positive and significant relationship among three predictors (NDF, ADF, CP) in terms of ME may be explained by the fact that contained M and S peripheral cells have high protein content and resist to both physical and enzymatic degradation (Rooney and Pflugfelder, 1986).

**CONCLUSION**

Acid detergent fiber content values of all species declined as maturity advanced from MF to HD. pH values declined from 4.98 to 4.31 as maturity of M, S and SSH advanced from MF stage to HD stage. Besides, the highest value of degradation parameters of a, b, (a+b) and EDM decreased for M. Although the lowest was found for SSH silages, ME values were not different between the species silages as maturity varying from MF to HD. Acid detergent fiber was found to be the best single predictor of effective dry matter degradability of SSH.

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