Iranian Journal of Applied Animal Science

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

Research Article

N. Kılıçalp[1*], H. Hızlı[2], M. Sümerli[3] and M. Avcı[4]

[1] Department of Animal Science, Faculty of Agriculture, Gaziosmanpaşa University, Tokat, Turkey
[2] Agricultural Research Institute of East Mediterranean, Adana, Turkey
[3] GAP International Agricultural Research and Training Center, Diyarbakır, Turkey
[4] Department of Crop Science, Faculty of Agricultural Science and Technologies, Ömer Halisdemir University, Nigde, Turkey

Received on: 10 Jul 2017
Revised on: 24 Aug 2017
Accepted on: 15 Sep 2017
Online Published on: Jun 2018

*Correspondence E-mail: numan.kilicalp@gop.edu.tr

Online version is available on: www.ijas.ir

ABSTRACT

This research was conducted to investigate *in situ* degradation characteristics of maize, sorghum and sorghum-Sudan grass hybrids. whole plant of maize (*TTM-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 ($EDMD_2$) 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 x 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 NH_3-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 stoves 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 analysied 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 elctrode 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 cm×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 320000 IU, vit D 75000 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;

DM (g/kg)= (initial weight-final weight) / (initial weight) × 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).

$$EDMD\ (1, 2, 3)= a + b \times (c/(c+k))$$

Where:
k= ruminialout flow rate, being k_1= 0.02/ h, k_2= 0.05/ h, k_3= 0.08/h.

EDMD (1, 2, 3) were determined by using those out flow rate figures as (k=0.02), (k=0.05) and (k=0.08) respectively. In addition to, metabolizable energy (ME MJ/kg) was calculated by the equation:

$$ME\ (MJ/kg)= 0.1073 \times DMD_{(48\ h)} + 2.27563$$

Where:
DMD: dry matter degradability for 48 h rumen incubation (Bhargava and Ørskov, 1987; Bhargava *et al.* 1988).

Statistical analysis

Statistical analysis was performed using the GLM procedure of SPSS Software-16 for Windows (SPSS, 2007). The model used for the analysis was:

$$Y_{ijkl}= \mu + \tau_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)}$$
i= 1, 2, 3; j= 1, 2, 3; k= 1, 2, 3; l= 1, 2, 3

Where:
μ: population mean for the variable.
τ_i: effect of the i^{th} species.
$\beta_{j(i)}$: effect of the j^{th} cultivar within the i^{th} species.
$\gamma_{k(ij)}$: effect of the k^{th} cutting stage within the j^{th} cultivar and i^{th} species.
$\varepsilon_{l(ijk)}$: usual error term.

CP, WL, NDF, ADF and pH values of cutting stage maturity and species of maize, sorghum and SSH silages were compared. Duncan’s range tests were used to evaluate significance of differences among the species, cultivars and cutting stages.

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 ML 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 of M, S and SSH did not change among species and cultivars except within S cultivars. 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).

Table 1 Effects of species, cultivars and cultivar × maturity cutting stage on washing loses and chemical content of silages

Items	N	Washing loses (%)	CP (%)	NDF (%)	ADF (%)
Species					
Maize	18	31.8[b]	6.1[b]	61.5[a]	46.0
Sorghum	18	28.7[a]	5.6[b]	64.8[b]	48.1
Sudan grass hybrids (SSH)	18	29.4[a]	4.4[a]	65.1[b]	47.9
SEM		0.237	0.079	4.61	0.314
P-values		< 0.0001***	< 0.0001***	< 0.0001***	0.140
Cultivars					
Maize (TTM-815)	9	31.0	6.0	62.3	46.5
DK-711	9	32.6	6.1	60.8	45.5
Sorghum (SS-506)	9	27.1[a]	4.4[a]	66.6	50.3[a]
FS-5	9	30.4[b]	6.8[b]	63.0	45.7[b]
SSH (P-988)	9	29.1	4.8[a]	66.0	48.7
Grazer (N2)	9	29.7	4.0[b]	64.2	47.1
SEM		0.167	0.0563	4.41	0.222
P-values		0.771	0.023*	0.542	0.627
Cultivar × maturity Cutting stage	54	< 0.000***	0.053	< 0.035*	< 0.001***

CP: crude protein; NDF: neutral detergent fibre and ADF: acid detergent fibre.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.001) and *** (P<0.0001).
SEM: standard error of the means.

Table 2 Effects of species, cultivars and cutting stage on chemical content of silages

Cutting stage	Species				SEM	P-value		
	N	Maize	Sorghum	SSH		Species	Cultivars	Cultivar × maturity Cutting stage × spp
	18		**Crude protein (%)**					
Mid-flowering	6	7.3[a]	4.9	5.0				
Milk-line	6	5.5[b]	5.8	4.3				
Hard dough	6	5.3[b]	6.2	4.0	1.03	< 0.0001	< 0.023	< 0.053*
	18		**NDF (%)**					
Mid-flowering	6	62.4[ab]	67.9	66.1				
Milk-line	6	63.3[b]	64.3	67.8				
Hard dough	6	58.8[a]	62.3	61.5	16.47	< 0.0001	> 0.542	< 0.035*
	18		**ADF (%)**					
Mid-flowering	6	51.0[a]	51.3[a]	51.3[a]				
Milk-line	6	45.7[b]	48.2[ab]	48.1[ab]				
Hard dough	6	41.2[b]	44.8[b]	44.2[b]	15.97	< 0.0001	> 0.627	< 0.001**
	18		**WL (%)**					
Mid-flowering	6	30.5[a]	26.0[a]	25.5[a]				
Milk-line	6	28.1[a]	31.0[b]	27.9[a]				
Hard dough	6	36.7[b]	29.3[ab]	34.8[b]	9.07	< 0.0001	> 0.771	< 0.0001***

SSH: Sudan grass hybrids; NDF: neutral detergent fibre; ADF: acid detergent fibre and WL: washing losses.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.001) and *** (P<0.0001).
SEM: standard error of the means.

pH values of silages

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 $EDMD_1$, $EDMD_2$ and $EDMD_3$ 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

Cutting stage					
Mid-flowering	**Milk line**	**Hard dough**	**N**	**SEM**	**P-values**
4.98^{a}	4.41^{b}	4.31^{b}	54	0.084	< 0.001**
Species					
Maize	**Sorghum**	**SSH**	**N**	**SEM**	**P-values**
4.59	4.56	4.53	54	0.131	> 0.953

SSH: Sudan grass hybrids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.001).

Table 4 Effects of species, cultivars and cultivar × maturity cutting stage on rumen degradation characteristics of silages

		Degradation characteristics										
Items	**N**	**a (g/kg)**	**b (g/kg)**	**a+b (g/kg)**	**c (h^{-1})**	**k1 (0.02/h)**	**k2 (0.05/h)**	**k3 (0.08/h)**	**EDMD1 (g/kg)**	**EDMD2 (g/kg)**	**EDMD3 (g/kg)**	**ME (MJ/ kg)**
Species												
Maize	18	285.98^{b}	447.86^{b}	733.83^{b}	0.011^{b}	2.74^{a}	6.84^{a}	10.94^{a}	287.61^{b}	286.65^{b}	286.43^{b}	7.77^{a}
Sorghum	18	258.70^{a}	454.66^{b}	713.37^{b}	0.011^{b}	2.83^{a}	7.06^{a}	11.31^{a}	260.44^{a}	259.37^{a}	259.15^{a}	8.44^{b}
Sorghum × Sudan grass hybrids	18	264.63^{a}	347.38^{a}	612.42^{a}	0.009^{a}	3.32^{b}	8.29^{b}	13.28^{b}	265.61^{a}	265.00^{a}	264.96^{a}	8.28^{ab}
SEM		6.391	12.969	13.464	0.0001	0.069	0.173	0.277	6.371	6.380	6.389	0.188
P-values		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivars												
Maize (TTM-815)	9	278.58	477.76	756.33	0.011	2.65	6.63	10.60	280.49	279.40	279.06	7.67
DK-711	9	293.38	417.96	711.33	0.010	2.82	7.05	11.29	294.73	293.90	293.80	7.87
Sorghum (SS-506)	9	243.76	448.84	692.60	0.011	2.92	7.29	11.68	245.54	244.43	244.20	8.53
FS-5	9	273.64	460.47	734.13	0.011	2.73	6.83	10.93	275.34	274.32	274.10	8.34
SSH (P-988)	9	261.78	370.84	633.44	0.010	3.19	7.99	12.78	262.88	262.15	262.15	8.46
Grazer N2	9	267.49	323.91	591.40	0.009	3.44	8.61	13.78	268.34	267.86	267.76	8.11
SEM		9.038	18.341	19.041	0.0004	0.098	0.245	0.392	9.010	9.023	9.036	0.266
P-values		< 0.769	< 0.257	< 0.297	< 0.613	< 0.250	< 0.256	< 0.255	< 0.772	< 0.770	< 0.770	< 0.694
Cultivar × maturity Cutting stage		< 0.0001	0.066	0.078	< 0.001	< 0.202	< 0.202	< 0.203	< 0.0001	< 0.0001	< 0.0001	< 0.531

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.

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

			Degradation characteristics										
Species	**Cutting stage**	**N**	**a (g/kg)**	**b (g/kg)**	**a+b (g/kg)**	**c (h^{-1})**	**k1 (0.02/h)**	**k2 (0.05/h)**	**k3 (0.08/h)**	**EDMD1 (g/kg)**	**EDMD2 (g/kg)**	**EDMD3 (g/kg)**	**ME (MJ/kg)**
Maize	MF	6	274.27^{a}	438.07^{ab}	712.33	0.0107^{b}	2.82	7.04	11.27	275.83^{a}	274.95^{a}	274.71^{a}	8.31^{b}
	ML	6	253.00^{a}	491.00^{b}	744.00	0.0118^{b}	2.70	6.76	10.81	255.08^{a}	253.92^{a}	253.49^{a}	7.29^{a}
	HD	6	330.67^{b}	414.50^{a}	745.17	0.0090^{a}	2.69	6.72	10.76	331.93^{b}	331.08^{b}	331.08^{b}	7.71^{ab}
P-value			< 0.0004	< 0.0576	< 0.4230	< 0.0010	< 0.4199	< 0.4267	< 0.4263	< 0.0004	< 0.0004	< 0.0004	< 0.0875
SEM			10.7	16.78	19.0	0.00043	0.07	0.18	0.29	0.64	10.67	10.69	0.28
Sorghum	MF	6	234.00	493.83	727.83	0.0125^{b}	2.77	6.91	11.05	236.12^{a}	234.85^{a}	234.49^{a}	8.36
	ML	6	278.73	433.50	712.23	0.0100^{a}	2.85	7.13	11.40	280.29^{b}	279.25^{b}	279.17^{b}	8.35
	HD	6	263.37	436.63	700.03	0.0105^{a}	2.87	7.16	11.47	264.91^{ab}	264.03^{ab}	263.80^{ab}	8.60
P-value			< 0.086	< 0.141	< 0.767	< 0.008	< 0.808	< 0.799	< 0.795	< 0.090	< 0.088	< 0.086	< 0.861
SEM			13.26	21.30	25.74	0.00046	10.66	0.27	0.44	12.23	13.22	13.25	0.36
Sorghum × Sudan grass hybrids	MF	6	229.57^{a}	353.30	584.10	0.0103	3.45	8.63	13.80	230.63^{a}	230.00^{a}	229.92^{a}	8.73^{b}
	ML	6	251.27^{a}	333.63	584.90	0.0092	3.48	8.70	13.92	252.13^{a}	251.60^{a}	251.56^{a}	8.21^{ab}
	HD	6	313.07^{b}	355.20	668.27	0.0085	3.03	7.57	12.12	314.07^{b}	313.42^{b}	313.39^{b}	7.93^{a}
P-value			< 0.0003	< 0.8612	< 0.1066	< 0.2033	< 0.1460	< 0.1447	< 0.1450	< 0.0003	< 0.0003	< 0.0003	< 0.0976
SEM			10.11	30.66	19.59	0.00066	0.16	0.41	0.66	10.04	10.08	10.11	0.22

MF: mid-flowering; ML: milk-line; HD: hard-dough; 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 $EDMD_2$ 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 higest 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 costant (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 cell 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.

Table 6 The effects of maturity stage of species on rumen degradation characteristics

Degradation Characteristics	Harvesting stage: Mid-flowering	Milk line	Hard dough	N	SEM	P-value
a (g/kg)	245.94[b]	261.0[b]	302.37[a]	54	5.71	< 0.0001***
b (g/kg)	428.4	419.38	402.11	54	10.82	0.61
a+b (g/kg)	674.76	680.38	704.49	54	11.31	0.53
c (h^{-1})	0.01033[a]	0.0099[ab]	0.0089[b]	54	0.00022	< 0.0001***
EDMD2 (k=0.05/h, g/kg)	246.60[b]	261.59[b]	302.85[a]	54	5.70	< 0.0001***
ME (MJ/kg)	8.47	7.95	8.08	54	0.111	0.142

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.

Table 7 Prediction of effective dry matter degradability using multiple regression

Species	Equations	P value	R^2	RMSE[1]
Maize	Y= 512.035 - 5.017 (ADF)	0.006**	0.390	1179
	Y= 687.967 - 3.544 (ADF) - 4.019 (NDF)	0.007**	0.480	1028
	Y= 683.725 - 4.342 (ADF) - 3.964 (NDF) + 6.235 (protein)	0.017*	0.507	1045
Sorghum	Y= 349.829 - 1.933 (ADF)	0.256	0.080	1275
SSH	Y= 551.395 - 6.112 (ADF)	0.002**	0.446	1179
	Y= 681.272 - 4.641 (ADF) - 3.117 (NDF)	0.003**	0.531	1064
	Y= 742.712 - 4.787 (ADF) - 2.816 (NDF) - 17.041 (protein)	0.002**	0.635	889
Equations	Y= $EDMD_{(k=0.05)}$			
Maize	Y= 453.524 - 4.99 (ADF)	0.012*	0.337	1412
	Y= 618.461 - 3.615 (ADF) - 3.768 (NDF)	0.020*	0.405	1351
	Y= 614.793 - 4.305 (ADF) - 3.721 (NDF) + 5.393 (protein)	0.047*	0.403	1405
Sorghum	Y= 350.064 - 1.924 (ADF)	0.257	0.080	1267
SSH	Y= 552.069 - 6.119 (ADF)	0.002**	0.448	1174
	Y= 681.481 - 4.653 (ADF) - 3.106 (NDF)	0.003**	0.533	1058
	Y= 742.754 - 4.798 (ADF) - 2.806 (NDF) – 16.995 (protein)	0.002**	0.636	884
Equations	Y= $ME_{(MJ\ kg^{-1})}$			
Maize	Y= 0.185 + 0.48 (ADF) + 0.024 (NDF) + 0.315 (protein) + 0.009 (ED2)	0.050*	0.494	0.441
	Y= 5.550 + 0.375 (protein)	0.011*	0.344	0.465
	Y= 4.114 + 0.409 (protein) + 0.005 (ED2)	0.015*	0.429	0.431
SSH	Y= 13.123 – 0.007 (a+b)	0.040*	0.237	0.583
	Y= 10.292 - 0.008 (a)	0.029*	0.264	0.340

SSH: Sudan grass hybrids.
** (P<0.001).
RMSE: root mean square error.

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 (a, b and (a+b)) of maize silage had the highest value among the species, while the lowest ruminal degradation characteristics results were determined for the SSH silages. Similar findings were reported by Resende *et al.* (2003).

Effective ruminal dry matter degradabilities ($EDMD_1$, $EDMD_2$ and $EDMD_3$) 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 EDMD decreased from the first harvesting stage to the third harvesting stage.

Current study results similiar 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 EDMD were determined 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.

ACKNOWLEDGEMENT

The authors would like to thank workers of GAP International Agricultural Research and Training Center and East Mediterranen Agricultural Research Institute for helping during the study.

REFERENCES

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Ashbell G., Weinberg Z.G., Azrieli A., Hen Y. and Horev B. (1991). Asimple system to study the aerobic deterioration of silages. *Canadian Agric. Eng*. **33,** 391-393.

Bal M.A., Coors J.G. and Shaver R.D. (1997). Impact of the maturity of maize for use as silage in the diets of dairy cows on intake, digestion and milk production. *J. Dairy Sci.* **80,** 2497-2503.

Beck S., Huthison S.A., GunterLosi T.C., Stewart C.B., Capps P.K. and Phillips J.M. (2007). Chemical composition and *in-situ* dry matter disappearance of sorghum × Sudan grass hybrids. *J. Anim. Sci.* **85,** 545-555.

Bhargava P.K. and Ørskov E.R. (1987). Manuel for the Use of Nylon Bag Technique in the Evaluation of Feedstuffs. Rowett Research Institute, Bucksburn, Aberdeen, Scotland.

Bhargava P.K., Ørskov E.R. and Walli T.K. (1988). Rumen degradation of straw. Selection and degradation of morphological component of barley straw by sheep. *Anim. Prod*. **47,** 105-110.

Buck G.R., Merrill W.G., Coppock C.E. and Slack S.T. (1969). Effect of recutting and plant maturity on kernel passage and feeding value of maize silage. *J. Dairy Sci*. **52,** 1617-1632.

Coors J.G., Albrecht K.A. and Bures E.J. (1997). Ear-fiil effects on yield and quality of silage corn. *Crop Sci. Abstr.* **37**, 243-247.

Fariani A., Warly L., Matsui T.A., Fujihara T. and Harumoto T. (1994). Rumen degradability of Italian Ryegrass (*Lolium multiflorum*) harvested at three different growth stages in sheep. *Asian-Australasian J. Anim. Sci.* **7,** 41-48.

Fisher D.S. and Burns J.C. (1997). Quality analysis of summer-annual forages. Effects of forage carbohydrate constituents on silage fermentation. *Agron. J.* **79,** 242-248.

Fu F., Guo C., Tang Q., Liu J. and Li W. (2011). Growth dynamics and optimal harvesting stage of two forage maize varieties. *Agric. Sci. China.* **10,** 220-227.

Gul İ., Demirel R., Kilicalp N., Sümerli M. and Kılıç H. (2008). Effect of crop maturity stages on yield, silage chemical composition and *in vivo* digestibility of the maize, sorghum and sorghum-Sudan grass hybrids grown in semi-arid condition. *J. Anim. Vet. Adv.* **7,** 1021-1028.

Hatew B., Bannink A., Van Laar H., De Jonge L.H. and Dijkstra J. (2016). Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. *J. Dairy Sci*. **99,** 1-15.

Hunt C.W., Kezar W. and Vinande R. (1989). Yield, chemical composition, and ruminal ferment ability of maize whole plant, ear, and stoves as affected by maturity. *J. Prod. Agric*. **2,** 357-366.

Johnson L.M., Harrison J.H., Hunt C., Shinners C., Doggett C.G. and Sapienza D. (1999). Nutritive value of maize silage as affected by maturity and mechanical processing: A contemporary review. *J. Dairy Sci*. **82,** 2813-2825.

Johnson L.M., Harrison J.H., Davidson D., Mahanna W.C., Shinners K. and Linder D. (2002). Corn silage management: Effects of maturity, inoculation and mechanical processing on pack density and aerobic stability. *J. Dairy Sci.* **85,** 434-444.

Khan S.H., Azım A., Sarwar M. and Khan A.G. (2011). Effect of maturıty on comparatıve nutrıtıve value and fermentatıon characterıstıcs of maıze, sorghum and mıllet sılages . *Pakistan J. Bot*. **43,** 2967-2970.

Khan S.H., Khan G.A., Sarwar M. and Azim A. (2007). Effect of maturity on production efficiency, nutritive value and *in situ* nutrients digestibility of three cereal fodders. *Int. J. Agric. Res*. **2,** 900-909.

Lai N.V. and Thu Huong N.T. (1999). Comparison of the *in sacco* rumen and washing loss methods to estimate the potential energetic value for livestock of leaves from tropical trees, shrubs and crop residues. *Livest. Res. Rural Dev*. **11,** 1-6.

Loveras J. (1990). Dry matter and nutritive value of four summer annual crop in North-West Spain (Galicia). *Grass and Forage Sci.* **45,** 243-248.

Marco M.S., Aelio O.N., Nomdedeu M. and Van Houtte S. (2002). Effect of maize crop maturity on silage chemical composition and digestibility (*in vivo*, *in situ* and *in vitro*). *Anim. Feed Sci. Technol.* **99,** 37-43.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC, USA.

Ørskov E.R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J. Agric. Sci. Camb.* **92,** 499-503.

Podkowka Z. and Podkowka L. (2011). Chemical composition and quality of sweet sorghum and maize silages. *J. Cent. Eur. Agric.* **12**, 294-303.

Pour A.H., Mohammad K., Gholamreza G., Mohammadreza E., Mohammadzadeh H. and Boroumandjaz M. (2012). Comparison of chemical and degradability characteristics in three sorghum silage varieties with maize silage using *in vitro* and *in situ* methods. Pp. 32-35 in Proc. 16th Int. Silage Conf. Hämeenlinna, Finland.

Rafıuddın A., Javed M., Jabbar K., Shahıd M.A., Jan M.Q.P., Khan S., Ramzan M.A. and Hamdullah M. (2016). Impact of flowerıng stage on nutrıtıve value, physıcal qualıty and digestibility of sılages made from cereal fodders**.** *Appl. Ecol. Envıron. Res.* **14,** 149-157.

Resende J.A.S., Pereira M.N., Von Pinho R., Henrique Fonseca A. and Da Silva A.R.P. (2003). Ruminal silage degradability and productivity of forage and grain-type sorghum cultivars. *Sci. Agric.* **60,** 457-463.

Rooney L.W. and Pflugfelder R.L. (1986). Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Anim. Sci*. **63,** 1607-1623.

Sonon R.N. and Bolsen K.K. (1996). Effects of cultivar and stage of maturity on agronomic characteristics, chemical composition and nutritive value of forage sorghum silages. *Adv. Agric. Res*. **5,** 1-17.

SPSS Inc. (2007). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc.

St. Pierre N.R., Bouchard R., St. Laurent G., Roy G.L. and Vinet C. (1987). Performance of lactating dairy cows fed silage from maize of varying maturities*. J. Dairy Sci*. **70,** 108-115.

Tabacco E., Borreani G., Valente M.E. and Peiretti P.G. (2004). Dry matter and water soluble carbohydrate content of Italian rye grass at affected by environmental factors. *Italian J. Agron*. **8,** 63-74.

Theurer C.B. (1986). Grain processing effects on starch utilization by ruminants. *J. Anim. Sci.* **63,** 1649-1662.

Undersnder D., Mertens D.R. and Thiex N. (1993). Forage Analysis Procedurs. National Forage Testing Association, Omaha, Nebraska.

Van Soest P.J. (1994). Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. **74,** 3583-3597.

Vecchiettini M., Cinti F. and Sandrini E. (2003). Effect of harvest stage on maize silage production. Pp. 303-306 in Proc. 12th Symp. Eur. Grassl. Fed. Pleven, Bulgaria.