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Effects of Sown Season and Maturity Stage on *In vitro* Fermentation and *In sacco* Degradation Characteristics of New Variety Maize Stover*

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ABSTRACT : The effects of seedtime and maturity stage on nutritive value of five maize stover varieties, including conventional maize (Kexiangyu 11, CM), fodder maize (Huqing 1, FM), high oil maize (Gaoyou 115, HOM), sweet maize (Kexiangtianyu 1, SM) and waxy maize (Kexiangluoyu 1, WM), were examined based on chemical composition, *in vitro* gas production and *in situ* incubation techniques. Maize stover was sampled at d 17 and d 30 after tasseling, and designated as maturity stage 1 and stage 2, respectively. The average dry matter (DM) organic matter (OM), crude protein (CP) and fiber contents were the greatest for HOM, SM and FM, respectively. CM had the highest *in vitro* organic matter disappearance (IVOMD) and volatile fatty acid (VFA) concentration. The highest ammonia nitrogen (NH_3-N) concentration in the incubation solution, and effective degradability of DM (ED_{DM}) and neutral detergent fiber (ED_{NDF}) were observed in SM. Advanced maturity stage increased ($p<0.05$) DM content, ED_{DM} and ED_{NDF}, but decreased ($p<0.05$) OM and CP contents, and decreased ($p<0.05$) *b* and *a*+*b* values, IVOMD and molar proportion of valerate in the incubation solution for maize stover. Maize sown in summer had greater ($p<0.05$) OM content, but lower DM, CP, neutral detergent fiber (NDF) and acid detergent fiber (ADF) content compared with maize sown in spring. Maize sown in summer had greater ($p<0.001$) IVOMD, NH_3-N concentration in the incubation solution and ED_{NDF}, but lower ($p<0.01$) ratio of acetate to propionate compared to maize sown in spring. The interaction effect of variety×seedtime was observed running through almost all chemical composition, *in vitro* gas production parameters and *in situ* DM and NDF degradability. The overall results suggested that SM had the highest nutrient quality, and also indicated the possibility of selecting maize variety and seedtime for the utilization of maize stover in ruminants. (**Key Words :** Maize Stover, Variety, Maturity Stage, Seedtime, Nutritive Value)

INTRODUCTION

Maize stover is one of the most abundant crop straw resources in the world, and is usually utilized as an important forage source for ruminants (e.g., dairy, cattle, sheep and goats) in many countries (Wilkinson and Toivonen, 2003). The nutritive value of maize stover is not only dependent upon the climatic conditions, cultivation practices such as fertilizing measurements, water management and storage methods, but also depends on chemical composition, and the relative proportions of plant organs (e.g. leaf blade, stem and leaf sheath) (Tang et al., 2006).

Physiological maturity stage of the crop and sown season are also important factors affecting the nutritive value of maize stover (Ettle and Schwarz, 2003; Tang et al., 2008; 2009). The number of leaves per plant and leaf/stem ratio decreases with the delay of harvest from physiological maturity to the dead ripe stage (Harika and Sharma, 1994). Moreover, with advancing maturity, the content of crude protein and soluble solid decreases, and the lignin and xylan content increases in the crop residues (Tolera et al., 1998; Pordesimo et al., 2005), thus reducing its digestibility (Russell, 1986).

In addition to the maturity stage, maize variety also affects the physical and chemical properties of maize stover

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(Harika and Sharma, 1994; Tang et al., 2008), and the feeding value and digestibility of maize stover or maize silage (Tolera et al., 1999; Ettle and Schwarz, 2003). Tang et al. (2008) reported that variety affected the proportion of morphological fractions of maize stover; waxy maize and fodder maize had greater proportions of stem and leaf blade, respectively, compared to conventional, high oil and sweet maize. Chemical composition of stover parts was also affected by the proportion of morphological fractions: leaf blade, stem and cob contributed over 0.75 of organic matter, crude protein, phosphorus and fiber in the whole plant of CM, FM, HOM, SM and WM varieties (Tang et al., 2009). Several studies showed that the stover of brown midrib varieties had greater digestibility than other maize varieties because of their lower lignin content (Oba and Allen, 1999; Tjardes et al., 2000). Therefore, difference in maize variety might affect the fermentation characteristics of stover.

During past decades, many new maize hybrids (such as SM, WM, HOM and FM) have been developed and planted all over the world. Fresh kernel of some corn hybrids (such as SM, WM, etc.) are processed for human consumption. Few studies, however, have been conducted to evaluate the effects of three factors, i.e. variety, maturity stage and seedtime, on the nutritive value of maize stover. This experiment was designed to investigate the comparative nutritive value of five varieties of maize stover harvested in two maturity stages when they were sown in spring and summer.

MATERIALS AND METHODS

This experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture (ISA), the Chinese Academy of Sciences, Changsha, China.

Maize varieties

Five maize varieties, namely conventional maize (Kexiangyu No.11, CM), fodder maize (Huqing No.1, FM), high oil maize (Gaoyou No. 115, HOM), sweet maize (Kexiangtianyu No. 1, SM) and waxy maize (Kexiangluoyu No. 1, WM) were used. Kexiangyu No.11, developed in ISA, is a conventional variety. Kexiangluoyu No. 1 and Kexiangtianyu No. 1 are short, early-maturing varieties released by ISA, through intensive selection for increasing glutinousness and sweetness. Gaoyou No. 115 is a variety containing a high oil concentration in its grain, and was developed in China Agriculture University in the 1996. Huqing No.1 is a hybrid variety used for ensiling.

Planting

The five maize varieties were planted under similar agronomic conditions on the same field at the experimental farm of ISA in southern China (28°12′N and 113°5′E; altitude 38 m). The soil type is classified as paddy soil. The prevailing climate at the experimental site is humid-temperate with an average annual rainfall of 1,361 mm, and the mean monthly temperatures between April and November 2005 were 16.8, 21.6, 25.9, 29.3, 28.7, 24.2, 18.5 and 12.5°C, respectively. The varieties were sown on the 22nd of April (spring), and the 9th of August (summer) 2005, with three replicated plots of 5 m×5 m for each variety. A mixed inorganic fertilizer was applied at sowing time (kg/ha: nitrogen, 60; phosphorus, 30; potassium, 50), and 100 kg urea/ha was applied when the plants reached knee height.

Harvesting and preparation

The tasseling dates were the 23rd, 23rd, 24th, 26th, and 28th of June, and the 3rd, 5th, 6th, 8th and 11th of October for CM, SM, WM, HOM and FM planted in spring and summer, respectively. Ten whole maize plants of each variety were harvested on d 17 (maturity stage 1) and d 31 (maturity stage 2) with 3 cm cutting height after tasseling per replicated plot for each variety in the two harvesting seasons. The kernels of ten maize plants were removed, and maize stover (including leaf, sheath, stem, husk and cob) were manually chopped to a length of 2 cm, dried at 65°C and ground through a 1-mm sieve prior for chemical analysis, *in vitro* and *in sacco* measurements.

Chemical analysis

Dry matter was determined by oven drying the sample at 105°C overnight, and ash was determined by igniting the samples in a muffle furnace at 550°C for 6 h. Crude protein content was calculated as 6.25×N which was obtained by the micro-Kjeldahl method. The NDF and ADF contents were determined according to the procedure described by Van Soest and Robertson (1985). A HP5890 gas chromatograph was used for VFA analysis as described by Vanzant and Cochran (1994). NH_3-N concentration in the incubation solution was determined as described by Weatherburn (1967).

In vitro gas production measurement

The samples were incubated *in vitro* with rumen fluid in calibrated glass syringes as described by Tang et al. (2006). Three rumen-fistulated goats (a local breed, 20±1.0 kg) were fed a total of 900 g dry maize stover and 900 g concentrate mix (containing per kg DM: 452 g maize grain, 360 g wheat bran, 126 g soybean meal, 16 g urea, 10 g salt, and 36 g trace element and vitamin premix), offered in equal portions twice daily. Rumen fluid was collected before feeding in the morning, and strained through four layers of gauze into a pre-warmed, insulated bottle. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2.

Approximately 200±10 mg dry weight of the samples were weighed in triplicate into syringes of 100 ml. Then the syringes were filled with 30±1 ml rumen fluid-buffer mixture. All the syringes were placed in a shaking water bath (DSHZ-300, Taicang, Jiangsu, China) with 50 movements per min at 39°C. Three syringes containing only incubation media were placed in the water bath and used as blanks to correct for the gas production due to the activity of the rumen fluid without any feed sample. Gas volume was recorded immediately after 2, 4, 6, 12, 24, 36, 48, 60 and 72 h of incubation. The result was fitted to the exponential equation described by Blümmel and Ørskov (1993): $GP = a+b(1-e^{-ct})$, where GP represents gas production at time t, and a, b and c are constants in the exponential equation, where a = the intercept of the gas production curve, b = the proportion of gas production during time (t), c = the rate of gas production of the b fraction. *In vitro* incubation was terminated at 72 h. The VFA content in the incubation solution was determined after 72 h of incubation by gas chromatograph. The residual solutions were filtered into pre-weighed filter crucibles, dried at 105°C for 24 h and ashed at 550°C for measuring *in vitro* organic matter disappearance (IVOMD).

In sacco DM and NDF degradation

Three goats (Liuyang black wether goat, a local breed, 35±2.0 kg) fitted with rumen cannula were used for *in sacco* trial. Each goat was fed a total of 450 g dry maize stover and 300 g concentrate mixture (containing per kg DM: 452 g maize grain, 360 g wheat bran, 126 g soybean meal, 16 g urea, 10 g salt, and 36 g trace element and vitamin premix) at the experimental farm of ISA, offered at 07:00 and 17:00 hrs. Animals could access water freely. Duplicate bags (6 cm×4 cm, pore size 37 μm, Taizhou, China) containing 2.5 g sample were incubated in the rumen of each goat for 2, 6, 12, 24, 36, 48, and 72 h after 20 days of adaptation to the diet. The bags were introduced into the rumen at 20:00 hrs and removed according to the required intervals. After removal, all nylon bags (including 0 h bags) were washed in a washing machine (XPB15-8006, Cixi, China) until the water was clear, and dried for 48 h at 60°C. The DM and NDF degradation data were fitted to the exponential equation: $p = a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979), where p is DM or NDF degradation (%) from the bags at time t. Since washing losses (A) were greater than the estimated rapidly soluble fraction (a), the lag time was estimated according to McDonald (1981) by fitting the model $p = A_0$ for $t \leq t_0$, and $p = A+B(1-e^{-Ct})$, for $t>t_0$. The degradation characteristics of the maize stover were defined as follows: A = washing loss (readily soluble fraction); $B = (A+B)-A_0$, representing the insoluble but fermentable material; C = the rate of degradation of B; the lag phase (L_{DM} and L_{NDF}) = $(1/C)\log_e[B/(A+B-A_0)]$. The equation of ED = $A+(BC/(C+kp))$ was used to calculate ED of DM (ED_{DM}) or NDF (ED_{NDF}) (McDonald, 1981), where A, B and C are constants representing the rapidly degradable DM or NDF fraction (A_{DM} and A_{NDF}, %), the slowly degradable DM or NDF fraction (B_{DM} and B_{NDF}, %) and the constant rate of DM or NDF degradation for fraction B (C_{DM} and C_{NDF}, per h), and kp is the passage rate of the particulate matter from the rumen at 3% per h (Ørskov et al., 1988).

Statistical analysis

The statistical analysis of data was done by analysis of variance with the software package of Statistical Analysis System (Release 8.2, SAS Institute Inc., Cary North Carolina, USA 2001) using the GLM procedure. The following model was used for the analysis of data:

$$Y_{ijkl} = \mu+R_i+V_j+S_k+M_l+V_j\times S_k+V_j \times M_l+S_k\times M_l+V_j\times S_k\times M_l+e_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, R_i = effect of replicate (i = 1, 2, or 3), V_j = effect of variety (j =1, 2, 3, 4 or 5), S_k = effect of seedtime (k =1 or 2), M_l = effect of maturity stage, and e_{ijkl} = the random residual error. The observed means of main effect factors were compared by Duncan's test. Statistical significances were considered to exist if $p<0.05$.

RESULTS

Chemical composition of maize stover

Effects of variety, maturity stage and sown season on chemical composition of maize stover were identified (Table 1). The DM and OM were greater ($p<0.001$) in HOM and WM than in FM and SM. Maize stover harvested in stage 2 or sown in spring had greater ($p<0.001$) DM content but lower ($p<0.01$) OM content than those harvested in stage1 or sown in summer. Two-way interactions ($p<0.001$) and a three-way interaction ($p<0.05$) for DM content were noted. Maturity stage interacted ($p<0.05$) with seedtime on OM content.

The greatest and the lowest CP content were observed in varieties of SM and CM which were 10 and 8 g/kg DM greater ($p<0.05$), and 6 and 9 g/kg DM lower ($p<0.05$) than those in WM and FM, respectively. Maize stover harvested in maturity stage1 and sown in spring had greater ($p<0.05$) CP content than those harvested in maturity stage 2 and sown in summer. Maize sown in spring had greater ($p<0.01$) NDF and ADF contents than those sown in summer. Maturity stage interacted with variety on NDF ($p<0.05$) and ADF ($p<0.001$). Seedtime interacted ($p<0.05$) with variety on ADF content and interacted ($p<0.01$) with maturity on NDF. A three-way interaction ($p<0.001$) for NDF and ADF

Table 1. Chemical composition of stover from different maize varieties sown at different seedtimes and harvested at two maturity stages (g/kg DM) [a]

Variety	Seedtime	Maturity stage	DM	OM	CP	NDF	ADF
CM	Spring	Stage1	135	921	89	618	389
		Stage2	253	919	87	606	383
	Summer	Stage1	157	928	78	594	359
		Stage2	204	943	79	600	346
FM	Spring	Stage1	156	928	103	640	379
		Stage2	251	898	97	573	344
	Summer	Stage1	160	936	86	542	343
		Stage2	176	930	82	608	361
HOM	Spring	Stage1	169	932	101	617	381
		Stage2	284	926	88	583	364
	Summer	Stage1	188	943	98	585	348
		Stage2	229	937	88	577	346
SM	Spring	Stage1	134	918	106	596	365
		Stage2	218	898	105	551	356
	Summer	Stage1	185	939	93	564	316
		Stage2	190	926	95	600	365
WM	Spring	Stage1	165	928	92	581	351
		Stage2	293	913	87	651	418
	Summer	Stage1	162	937	104	560	314
		Stage2	241	942	75	590	340
		SEM	3.27	6.26	5.82	15.55	9.35
Significance							
V			***	*	*	0.22	0.10
S			***	***	**	**	***
M			***	**	*	0.55	0.07
V×M			***	0.07	0.18	*	***
V×S			***	0.62	0.29	0.26	*
M×S			***	*	0.56	**	0.07
V×M×S			*	0.65	0.40	**	**

[a] V = Variety; M = Maturity stage; S = Seedtime; V×M = Interaction between variety and maturity stage; M×S = Interaction between maturity stage and seedtime; V×M×S = Interaction between maturity stage, variety and seedtime. * p<0.05; ** p<0.01; *** p<0.001.

contents was also observed.

In vitro fermentation characteristics

The effects of seedtime, maturity stage and variety on *in vitro* gas production parameters and IVOMD of maize stover are presented in Table 2. The lowest IVOMD was noted in WM (p<0.001). Maize sown in summer had greater (p<0.001) IVOMD than that sown in spring. Intercept of gas production curve (*a*) of maize stover was greater (p<0.001) for FM than for CM and HOM, and for maize sown in spring than for maize sown in summer. The *b* value was greater (p<0.05) for WM, maize sown in summer and maize stover harvested at stage 1 than for the other varieties, maize sown in spring and maize stover harvested at stage 2, respectively. HOM and WM had greater (p<0.001) gas production rate than FM and CM. Potential gas production (*a*+*b*) (p<0.01) and IVOMD (p<0.05) decreased with the increase of maturity. FM and WM had greater (p<0.001) potential gas production than CM, HOM and SM.

Variety interacted with maturity stage on *a* (p<0.001), *b* (p<0.001), *c* (p<0.01) and IVOMD (p<0.05), and with seedtime on *a* (p<0.001), *c* (p<0.001), *a*+*b* (p<0.05) and IVOMD (p<0.01). A two-way interaction was observed between maturity stage and seedtime for *a* (p<0.001) and *b* (p<0.01). A three-way interaction was also observed between variety, maturity stage and seedtime for all parameters (p<0.01, except for *a*+*b*).

Both variety and seedtime had a significant effect on NH_3-N concentration in the solution after 72 h of incubation. SM had the greatest NH_3-N content, which was greater (p<0.05) than that of HOM, FM and WM. Ammonia nitrogen content in incubation solution was greater (p<0.001) for maize sown in summer than in spring. Seedtime interacted (p<0.001) with variety on NH_3-N content.

Table 2. *In vitro* fermentation characteristics of different varieties of maize stover sown at different seedtimes and harvested at two maturity stages [a]

Variety	Seedtime	Maturity stage	*a*, %	*b*, %	*c*, h	*a+b*, %	IVOMD, %	NH_3-N mmol/L	VFA mmol/L	Molar proportion of VFA, %						A :P
										Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	
CM	Spring	Stage1	1.42	52.0	0.066	53.4	68.8	7.07	55.5	51.1	24.9	5.23	11.4	3.10	4.30	2.06
		Stage2	5.88	43.1	0.044	49.0	56.7	10.40	56.5	47.6	24.8	6.16	14.3	4.82	2.39	1.95
	Summer	Stage1	8.79	47.7	0.045	56.5	63.8	24.28	75.0	50.2	28.1	4.78	10.3	4.68	2.00	1.79
		Stage2	9.19	45.2	0.05	54.3	66.0	22.52	68.0	49.7	28.7	5.05	11.4	3.30	1.93	1.74
FM	Spring	Stage1	10.8	46.0	0.039	56.8	59.0	6.98	50.7	48.3	28.9	6.24	11.4	3.13	2.09	1.68
		Stage2	10.2	46.3	0.037	56.5	60.0	5.91	55.6	50.8	27.7	6.00	10.9	2.80	1.79	1.84
	Summer	Stage1	16.4	38.2	0.044	54.6	71.0	10.60	44.7	45.6	26.0	6.06	14.7	5.01	2.66	1.76
		Stage2	7.15	51.6	0.06	58.8	61.4	10.49	43.0	45.5	25.9	6.22	14.6	5.27	2.58	1.76
HOM	Spring	Stage1	3.44	53.1	0.065	56.5	46.3	8.69	65.7	47.5	24.5	5.59	16.4	3.86	2.22	1.94
		Stage2	6.24	47.9	0.075	54.1	70.3	6.90	51.4	54.4	23.9	5.28	11.6	2.98	1.90	2.28
	Summer	Stage1	6.35	48.6	0.052	55.0	68.9	23.32	73.3	43.9	31.2	5.52	11.9	4.92	2.52	1.41
		Stage2	4.91	41.3	0.052	46.2	59.3	22.53	58.8	42.5	32.6	6.18	11.5	5.01	2.29	1.31
SM	Spring	Stage1	4.48	49.3	0.060	53.8	62.0	10.14	40.1	44.8	25.8	6.86	14.6	5.21	2.72	1.74
		Stage2	9.63	40.5	0.054	50.2	61.0	10.85	30.3	45.6	26.4	6.42	14.2	4.91	2.50	1.73
	Summer	Stage1	9.26	45.2	0.050	54.5	66.6	25.05	59.2	47.7	30.4	4.73	11.4	3.45	2.26	1.57
		Stage2	7.66	44.7	0.045	52.4	58.8	22.83	69.1	49.9	28.0	5.24	11.1	3.78	1.93	1.78
WM	Spring	Stage1	8.27	52.8	0.051	61.1	54.3	7.38	64.0	45.8	22.7	6.27	17.1	5.41	2.71	2.04
		Stage2	4.20	52.9	0.049	57.1	43.0	7.45	68.0	52.0	21.9	5.46	14.4	4.02	2.29	2.38
	Summer	Stage1	6.50	51.9	0.076	58.4	57.4	10.52	42.1	49.1	25.2	5.03	14.6	3.81	2.26	1.97
		Stage2	9.06	49.7	0.062	58.8	68.3	10.13	42.7	44.2	26.8	6.48	15.3	4.74	2.52	1.65
		SEM	0.99	1.67	0.0035	1.84	2.69	0.952	4.68	2.49	1.36	0.235	0.710	0.326	0.210	0.090
V			***	***	***	***	***	***	***	**	***	*	***	0.12	0.34	***
S			***	*	0.70	0.91	***	***	0.08	***	***	**	**	*	0.17	***
M			0.73	**	0.19	**	*	0.35	0.21	0.08	0.81	0.14	0.11	0.50	*	0.25
V×M			***	***	**	0.11	*	0.61	0.11	*	0.80	0.64	***	0.74	0.24	0.51
V×S			**	0.24	***	*	**	***	***	***	***	***	***	***	***	***
M×S			***	**	0.15	0.46	0.24	0.13	0.94	***	0.46	**	*	0.32	0.06	*
V×M×S			***	**	***	0.26	***	0.12	0.27	***	0.33	*	**	***	0.19	**

[a] IVOMD = *In vitro* organic matter disappearance; *a* = The intercept of gas production curve; *b* = The proportion of gas production during time (t); *c* = The rate of gas production of the '*b*' fraction. A:P = The ratio of acetic to propionate. SEM = Standard error of mean.
V, M, S, V×M, M×S and V×M×S as explained in Table 1. * p<0.05; ** p<0.01; *** p<0.001.

Maize varieties differed (p<0.001) in the concentration of total VFA of the incubation solution according to the following decreasing order: CM>HOM>WM>SM>FM. Variety interacted (p<0.001) with seedtime on total VFA content. Molar proportions of acetate from CM, isobutyrate from FM and butyrate from WM or maize sown in spring were greater (p<0.01) than for the other varieties or maize sown in summer. Molar proportion of propionate was greater (p<0.001) for HOM than for CM and WM, and for maize sown in summer than for maize sown in spring. Maize sown in summer or stover harvested in stage1 had greater (p<0.05) molar proportions of isovalerate and valerate than maize sown in spring or stover harvested in stage2. The ratio of acetate to propionate (A:P) of the five varieties was decreased (p<0.001) in the following order: WM>CM>FM>HOM>SM. Maize sown in summer had greater (p<0.001) A:P than maize sown in spring.

Variety interacted with maturity on molar proportions of acetate (p<0.05) and butyrate (p<0.001), and interacted (p<0.001) with seedtime on total VFA content, A:P and VFA molar proportions. There was a maturity stage×seedtime interactive effect on molar proportions of acetate (p<0.001), isobutyrate (p<0.01), and butyrate (p<0.05) and for A:P (p<0.05). A three-way interaction was observed between variety, maturity stage and seedtime for molar proportions of acetate (p<0.001), isobutyrate (p<0.05), butyrate (p< 0.01), and isovalerate (p<0.001), and for A:P (p<0.01).

In sacco degradability characteristics

Dry matter degradation parameters are shown in Table 3. The value of A_{DM} decreased (p<0.001) in the order: FM> SM>WM>CM>HOM. WM had a greater (p<0.001) B_{DM} value than FM, SM and HOM. HOM had greater (p<0.001) C_{DM} and L_{DM} values than SM, FM and WM. The ED_{DM} value was greater (p<0.001) in SM than in CM and WM whereas FM had the intermediate ED_{DM} value. Maize stover was greater (p<0.05) in A_{DM}, B_{DM} and ED_{DM} values, but lower (p<0.01) in C_{DM} value for maize sown in summer than for maize sown in spring. Advanced maturity stage increased (p<0.001) A_{DM} value and decreased (p<0.05) L_{DM} value, respectively. Variety interacted (p<0.05) with maturity stage (except for B_{DM} value) and with seedtime (except for ED_{DM} value) on all DM degradation parameters. Maturity stage interacted (p<0.001) with seedtime on A_{DM}

Table 3. Dry matter and neutral detergent fiber degradation characteristics of different varieties of maize stover sown at different seedtimes and harvested at two maturity stages [a]

Variety	Seedtime	Maturity stage	A_{DM} %	B_{DM} %	C_{DM} h^{-1}	L_{DM} h	ED_{DM} %	A_{NDF} %	B_{NDF} %	C_{NDF} h^{-1}	L_{NDF} h	ED_{NDF} %
CM	Spring	Stage1	33.3	43.2	0.0223	0.491	51.4	8.43	59.2	0.0235	0.609	34.0
		Stage2	34.3	38.4	0.0216	0.731	49.9	5.54	50.1	0.0298	0.622	29.9
	Summer	Stage1	36.7	40.7	0.0251	0.605	54.9	7.87	62.3	0.0232	0.602	34.5
		Stage2	36.2	40.2	0.0306	0.814	55.9	7.74	61.2	0.0262	0.295	36.3
FM	Spring	Stage1	36.5	27.2	0.0217	0.289	47.6	7.10	40.1	0.0264	0.230	25.2
		Stage2	40.6	34.1	0.0311	0.674	57.5	18.61	47.5	0.0310	1.039	41.8
	Summer	Stage1	41.3	41.4	0.0217	0.653	58.3	9.78	60.8	0.0243	0.023	37.4
		Stage2	37.9	40.5	0.0183	0.302	53.0	12.17	55.6	0.0197	0.000	34.7
HOM	Spring	Stage1	31.2	35.2	0.0307	1.042	48.1	8.98	57.4	0.0199	1.369	30.0
		Stage2	35.9	40.4	0.0220	1.201	52.3	10.67	60.2	0.0183	2.249	31.8
	Summer	Stage1	34.5	38.2	0.0269	1.249	51.8	3.32	52.8	0.0322	0.626	30.1
		Stage2	37.5	31.4	0.0272	1.001	51.9	7.98	48.4	0.0261	0.200	30.2
SM	Spring	Stage1	35.7	37.9	0.0225	1.003	51.5	11.50	58.1	0.0185	1.538	32.0
		Stage2	41.1	29.5	0.0301	0.368	55.7	12.91	45.6	0.0246	1.024	32.6
	Summer	Stage1	37.9	42.2	0.0237	0.834	56.1	5.66	62.3	0.0240	0.116	34.1
		Stage2	37.2	46.2	0.0208	0.435	55.6	12.92	58.5	0.0283	0.807	40.4
WM	Spring	Stage1	36.5	43.4	0.0266	1.600	55.8	2.07	68.8	0.0274	0.246	34.6
		Stage2	29.4	45.6	0.0192	0.710	46.6	8.93	51.1	0.0268	1.098	32.2
	Summer	Stage1	39.6	42.0	0.0167	0.855	54.0	9.60	51.9	0.0224	0.487	31.4
		Stage2	39.4	41.7	0.0182	0.177	54.3	12.42	50.2	0.0258	0.096	35.6
		SEM	0.24	2.60	0.00153	0.197	1.02	0.347	2.12	0.00173	0.1951	1.49
V			***	***	***	***	***	***	0.07	0.42	***	***
S			**	***	*	**	0.18	***	0.10	0.43	***	**
M			***	***	0.78	0.87	*	0.51	**	0.06	0.07	**
V×M			***	***	0.55	**	**	***	0.13	**	0.40	**
V×S			***	***	***	***	*	0.30	***	***	***	*
M×S			0.32	***	0.62	0.90	0.41	*	0.40	0.06	**	0.68
V×M×S			***	***	*	***	0.36	***	*	0.10	***	***

[a] A_{DM} = Readily soluble fraction of DM; B_{DM} = Insoluble but fermentable material of DM; L_{DM} = Lag time of DM; c_{DM} = The rate of degradation of B_{DM} fraction of DM; ED_{DM} = Effective DM degradability. A_{NDF} = Readily soluble fraction of NDF; B_{NDF} = Insoluble but fermentable material of NDF; L_{NDF} = Lag time of NDF; c_{NDF} = The rate of degradation of B_{NDF} fraction; ED_{NDF} = Effective NDF degradability.
V, M, S, V×M, M×S and V×M×S as explained in Table 1. * p<0.05; ** p<0.01; *** p<0.001.

and ED_{DM}. We also noted a three-way interaction ($p<0.05$) for A_{DM}, B_{DM}, C_{DM} and ED_{DM}.

The value of A_{NDF} decreased in the following order for the five maize varieties: FM>SM>WM>HOM>CM (Table 3). SM and HOM had longer ($p<0.001$) lag time than the other maize varieties. The lowest ED_{NDF} was observed in HOM which was lower ($p<0.001$) than the other four maize varieties. The values of A_{NDF} and L_{NDF} were greater ($p<0.01$) for maize sown in spring than in summer. Maize sown in summer had greater ($p<0.01$) ED_{NDF} value than that sown in spring. Maturity stage increased ($p<0.01$) A_{NDF} and ED_{NDF} values, but decreased ($p<0.01$) B_{NDF} value. Variety interacted ($p<0.01$) with maturity stage on A_{NDF}, C_{NDF} and ED_{NDF} values, and with seedtime in all parameters ($p<0.001$; for ED_{NDF}, $p<0.05$). A three-way interaction ($p<0.001$; for B_{NDF}, $p<0.05$) on A_{NDF}, B_{NDF}, L_{NDF} and ED_{NDF} values was also observed.

DISCUSSION

Effect of variety

Dry matter content of maize stover is a major factor influencing maize silage quality (Savoie et al., 2002). At both stages of harvesting, most of the maize stover in the present study had lower DM content (Table 1) than recommended for proper conditions of ensiling (Jones and Jones, 1995). The results indicated that the five genotypes of maize stover used in this study were unsuitable for ensiling (except for CM, FM and SM) when they were harvested before 31 d of tasseling. The new maize varieties

(FM, HOM, SM and WM) had higher CP contents than CM, which were in agreement with the results of previous studies (Jaster et al., 1983; Mustafa et al., 2004). This might indicate that the new maize varieties had higher nitrogen enrichment ability and could supply more CP for ruminants compared with the CM variety. Fiber content of maize stover (NDF ranged from 542-651 g/kg DM, ADF ranged from 316-418 g/kg DM) in this study was close to the findings of Cone et al. (2008), but lower than the results of Veribič et al. (1995) and Tolera et al. (1999).

A significant systematic effect of maize variety on *in vitro* fermentation characteristics was in agreement with the results of Cone et al. (2008), who also found unsystematic effects of the maize types Stay Green and Dry Down on the *in vitro* degradation of ear-free stover. The five maize varieties differed in ripening, but the fixed harvest date selected might be responsible for the variance in this study.

Although ammonia nitrogen arises from the degradation of CP, the concentration of ammonia nitrogen in the *in vitro* fermentation solution and the CP content of maize stover in this study contradicted this concept. This phenomenon also existed among different morphological fractions of maize stover (Tang et al., 2008; 2009). So, the difference of ammonia nitrogen concentration among the five varieties could not be interpreted fully from CP content. Differences in the CP degradability of maize stover or in the usage of NH_3-N by ruminant microorganisms might be responsible for this difference.

Volatile fatty acids have an important role in energy absorption and metabolism in ruminants. Ettle and Schwarz (2003) found that there were significant differences in the molar proportions of VFA among different maize varieties. In the present study, we detected significant differences in the molar proportion of VFA in the incubation solution among different maize varieties. This might imply that different maize varieties differed in energy utilization by ruminants. The observation that acetate molar proportion in the incubation solution of CM was greater than for the other varieties was in agreement with the results of Akay and Jackson (2001). New maize varieties (except for WM) had a higher molar proportion of propionate, which is a precursor for gluconeogenesis, than CM, which might imply new maize varieties had higher energy retention efficiency than CM in ruminants.

The effective degradability of DM and NDF were higher, but the degradation rate of the slowly degradable fraction and lag time of SM in this study were lower than reported for sweet corn residues by Mustafa et al. (2004). Also, Jaster et al. (1983) noted a DM digestibility of sweet corn residue similar to that of Mustafa et al. (2004), which might indicate that DM digestibility of SM in this study was higher than that of sweet corn residues. Difference of harvest date and variety might be responsible for this variation. Variety effect on *in situ* DM and NDF degradation was statistically significant and unsystematic, which was in agreement with the results of Tolera et al. (1999), Akbar et al. (2002) and Cone et al. (2008). Overall results of this study showed that the nutritive value of maize stover could be improved by breeding.

Effect of maturity stage

The observation that advanced maturity stage increased the content of DM, but decreased the CP content of maize stover was consistent with previous studies (Tolera et al., 1998; Ettle and Schwarz, 2003; Tang et al., 2009). Wang et al. (2006) also noted decreased CP content in rice straw with increasing stage of maturity. Tang et al. (2008) reported that over 50% of CP of whole-plant maize stover was contributed by leaf blade, on average. This suggests that the decrease of CP content in leaf blade with the delay of harvesting might account for the overall CP decrease of maize stover.

Decrease in NDF and ADF content with increased maturity for CM, FM and HOM in the present study was inconsistent with previous studies (Russell, 1986; Harika and Sharma, 1994; Tolera and Sundstøl, 1999). Results of Cone et al. (2008) showed that fiber content (aNDF and ADF) was higher for maize stover harvested at an earlier stage than when harvested at an advanced stage, which supported the present results. Miron et al. (2006) also reported that NDF content of sorghum was higher for sorghum cut at early heading stage than that cut at soft dough stage. This variation in results from different studies probably arose from the difference in harvesting date and maize variety. Varieties of CM, FM and HOM are late-maturing varieties compared to WM and SM. Decreased fiber content of these maize varieties with increasing maturity stage might imply that the maturity stage in the present study was a non-fibrous matter accumulation period, and a deferred harvest date for CM, FM and HOM stover might be more appropriate when harvested as feed for ruminants.

The values of IVOMD and $a+b$ of maize stover decreased with the increase of maturity stage and were consistent with the results of Tolera et al. (1998) and Tolera and Sundstøl (1999), who noted the decline of $a+b$ value at the advanced maturity. Cone et al. (2008) also reported that maximum gas production of maize stover decreased with the increase of DM content (i.e. the increase of maturity stage). Maize stover harvested at stage 2 had greater readily soluble fractions of DM and NDF, and greater effective degradability of NDF than when harvested at stage 1, which differed from the results of Tolera and Sundstøl (1999), Akbar et al. (2002) and Cone et al. (2008). The inconsistency between the present study and previous studies was likely related to differences of maize variety and harvested date. Yan et al. (2005; 2006) reported that the *in situ* degradability of DM and NDF for high oil maize and silage maize type increased as maize stover was harvested from 1/2 to 4/4 mile line, which was consistent with the present study. Also, Givens and Deaville (2001) showed that *in vivo* digestibility of DOMD and NDF quadratically increased for newer genotypes of maize silage, but linearly decreased for old genotypes of maize silage with the increase of DM content.

Effect of sowing season

Available literature is limited for assessing the influence of sowing season on fodder quality (Reddy et al., 2003). This study detected statistical significance in chemical composition between two planting seasons (Table 1). Content of NDF in forage is one of the main factors influencing dry matter intake. Maize stover had lower NDF content when it was sown in summer compared to that sown in spring, which might relate to the lower temperature and rainfall during the period of silking and ear-filling for maize sown in summer compared to spring. Crasta et al. (1997) reported that maize forage planted in a dry season or in a cool location had lower fiber content than that planted in a wetter season or warm location. Lower NDF content resulted in a higher IVOMD. Maize stover had lower CP content when it was sown in summer, which might be related to low temperature in the silking and ear-filling period which restricted the utilization of N (Boonchoo et al., 1998).

In vitro fermentation and *in situ* degradability showed moderate variation resulted from environmental conditions, as indicated by significant effects of sowing season (Tables 2 and 3). Correlation analysis results revealed that the intensity of change in A_{DM} and ED_{DM} was strongly associated with fiber content (Figure 1) and IVOMD was negatively related to NDF content (r^2 = -0.49, p = 0.03, n = 20). These findings generally agreed with the work of Russell (1986), Crasta et al. (1997) and Givens and Deaville (2001). Maize stover growing in the spring had greater fiber content (NDF and ADF) than that growing in summer, which might indicate that the quality of the latter was better than the former. The NH_3-N concentration in the incubation solution was not synchronous with the CP content of maize stover obtained from the two growing seasons. This might be ascribed to differences in CP degradability. Higher NH_3-N concentration in the incubation solution for stover growing in summer indicated that protein in these feeds was easily degraded in the process of *in vitro* fermentation. Apart from the NH_3-N, propionate concentration in the incubation solution was also remarkably higher for stovers growing in summer than for stovers growing in spring, which might be explained by the higher non-structure carbohydrate content of the former compared to the latter. This might imply that the stover growing in summer could supply more efficient energy for ruminants than stover growing in spring. Generally, the climate conditions (such as temperature, rainfall etc.) in the same season is similar between years. Ørskov et al. (1990) suggested that the differences in potential degradability of cereal straws were consistent between years. The current results implied that the influence of sowing season on nutritive value of maize stover might be more significant than the effect of years in the same location. Because the present study was only based on the data of one year and one location, it might be necessary to validate the effects of variation in season on the nutritive value of maize stover with more studies.

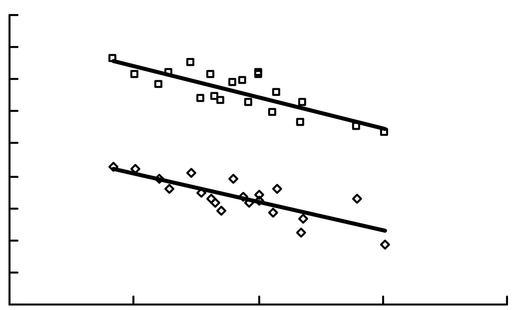

Figure 1. Correlations between NDF content and *in situ* DM degradability (◊: A_{DM}, Y = -0.0888X+89.163, R^2 = 0.6256; □: ED_{DM}, Y = -0.0954X+109.55, R^2 = 0.6408).

CONCLUSION

Data from this study demonstrated significant differences in chemical composition and *in vitro* fermentation parameters of maize stover among five varieties and between two maturity stages and two seedtimes. The OM and CP contents in maize stover decreased with increasing maturity stage. The contents of CP, NDF and ADF were affected by seedtime. The maize variety and seedtime and their interaction influenced *in vitro* fermentation parameters and *in situ* DM and NDF degradability. The *in situ* DM degradability was mainly related to NDF content of stover. Based on the chemical composition and *in vitro* and *in vivo* degradability, the SM maize variety had the highest nutrient quality. The information gathered from this study suggests that new genotype maize varieties or maize sown in summer have higher nutritive value than conventional varieties or that sown in spring, and the seedtime should be considered when these corn stovers are utilized in developing feeding strategies for ruminants.

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