Journal of Animal and Feed Sciences, 15, 2006, 505–515

A comparison of *in vitro* fermentation characteristics of different botanical fractions of mature maize stover*

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(Received 21 December 2005; revised version 15 February 2006; accepted 5 July 2006)

ABSTRACT

An *in vitro* cumulative gas production technique was used to evaluate the nutritional characteristics of different botanical fractions of mature maize stover (variety Kexiang No.11). Whole maize stover was separated into eight fractions: leaf blade, leaf sheath, whole stem, upper stem, lower stem, cob, husk, and tassel. The results showed that there were significant ($P<0.05$) variations among different botanical fractions in *in vitro* gas parameters (e.g., the theoretical maximum volume of gas production, the rate constant of gas production, and fermentation lag time). Husk had the highest theoretical maximum gas production and rate constant, while leaf sheath had the longest lag time. The hemicellulose content was positively related ($P<0.05$) with cumulative gas production at 24, 36 and 48 h of incubation and with the theoretical maximum gas production. The hemicellulose-to-crude-protein ratio was positively related ($P<0.05$) to the rate constant of gas production. *In vitro* gas production showed a negative but not significant ($P>0.05$) relationship with acid detergent fibre (ADF) and neutral detergent soluble (NDS) contents of the morphological fractions.

KEY WORDS: maize stover, gas technique, ruminants

INTRODUCTION

Agro-industrial by-products and crop residues are becoming the major feed resources for ruminants (e.g., dairy, cattle, sheep and goats) in regions where fresh pasture supply is limited and seasonal. The nutritive value of these products varies

* Supported by the project “Knowledge Innovation, kzcx2-sw-323” and “National Scientific and Technological Project, 2001-BA-508-B03”
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significantly, however, depending on the variety, climatic conditions, morphological composition and cultivation practices such as fertilizer use, water management, stage of maturity, and storage methods (Sannasgala and Jayasuriya, 1987). Several researchers have shown that variation in nutritive value exists between different botanical fractions of rice, wheat, and oat straw (Sannasgala and Jayasuriya, 1987; Shand et al., 1988; Nakashima and Ørskov, 1990). In particular, Tan et al. (1995) found significant differences in nutritive value among different morphological fractions of wheat straw. Apart from these variations in nutritive value, ruminants can select different fractions while feeding, (especially sheep and goats), which makes it difficult for nutritionists to formulate a supplementary diet to meet the nutrient requirements of these animals. Characterizing the nutritional value of the botanical fractions of agro-by-products will enable nutritionists to develop more precise feeding strategies for ruminants.

While maize stover is one of the major by-products used for ruminant feed, very little information is available on the nutritional characteristics of its different botanical fractions, i.e. the leaf blade, leaf sheath, stem, cob, husk, and tassel. The *in situ* nylon bag degradation and the *in vitro* cumulative gas production techniques have both been extensively used to evaluate the nutritional characteristics of roughages for ruminants (Dhanoa, 1988; Menke and Steingass, 1988). *In vitro* fermentative gas production has been proven to be positively related to intake and microbial protein synthesis (Krishnamoorthy et al., 1991a; Blummel and Ørskov, 1993; Hillman et al., 1993). Thus the measurement of *in vitro* gas production is often used as an indicator for assessing the potential nutritive value of a feedstuff. To understand the nutritional characteristics of maize stover, an *in vitro* gas production technique was used in the current study to evaluate the *in vitro* fermentation characteristics of different botanical fractions of mature maize stover and to define the relationships between *in vitro* fermentation characteristics and chemical composition.

MATERIAL AND METHODS

Sample preparation

The experiment was conducted at the experimental farm of the Institute of Subtropical Agriculture in southern China (28°12′N and 113°5′E; altitude 38 m). The maize, variety Kexiang No.11 with 16 leaves (HPAAC, 2004), was sown in a 0.3 ha paddock with a soil pH of 6.1 on March 24, 2003. The plant density was 6 seedlings/m^2. A mixed inorganic fertilizer was applied at sowing time (kg/ha: calcium superphosphate, 750; potassium chloride, 360; ammonium bicarbonate, 1500) and also when the plants reached knee height (100 kg urea/ha). After the maize grain was harvested on July 27, 2003, the maize stover of 50 plants was cut and taken to the laboratory for dissection.

Ten whole maize stovers were manually cut to a length of 2-cm. The other 40 plants were carefully separated by hand into six botanical fractions: the leaf blade, leaf sheath, whole stem, cob, husk, and tassel. The whole stem was cut from the middle of the eighth and the ninth node, and separated into two parts-upper stem and lower stem. All samples from the different botanical fractions were cut to a length of 2-cm length, dried at 65°C in an oven and then milled (DF-2, Changsha Instrument Factory, China) through a 1-mm sieve prior to chemical analysis and *in vitro* gas production measurements.

Chemical analysis

All maize stover samples were analysed for dry matter (DM), crude protein (CP), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose (HC) and neutral detergent soluble (NDS). The mixed diet was assayed for CP, NDF, ADF, calcium (Ca) and phosphorus (P). DM, OM, CP, Ca and P were determined following AOAC procedures (1990). NDF and ADF were analysed using the method of Van Soest et al. (1991). HC and NDS were calculated according to the equations: HC% = NDF%-ADF%, NDS% = 100%-NDF%.

Measurement of in vitro gas production

In vitro cumulative gas production was determined using the method described by Menke and Steingass (1988). Rumen fluid was collected from three ruminally fistulated Chinese Liuyang Black goats (body weight 14.2 kg±0.5) before the morning feed. The rumen fluid was mixed using equal volumes of fluid from the three goats. Three experimental goats were fed a mixed diet of maize stover and concentrate (1:1, w/w). Ingredient and chemical composition of the mixed diet for these goats is listed in Table 1. The goats were offered equal amounts (up to 5% refusal) of the diet at 07.00 and 19.00 h daily. The rumen fluid mixture was filtered through three layers of cheesecloth into a pre-warmed, insulated bottle and taken to the laboratory. The fluid was then mixed (1:2, v/v) with an anaerobic buffer and a mineral solution containing, per litre, g: 8.75 $NaHCO_3$, 1.00 NH_4CO_3, 1.43 Na_2HPO_4, 1.55 KH_2PO_4, 0.15 $MgSO_4{\cdot}7H_2O$, 0.52 Na_2S, 0.017 $CaCl_2{\cdot}2H_2O$, 0.015 $MnCl_2{\cdot}4H_2O$, 0.002 $CoCl_3{\cdot}6H_2O$, 0.012 $FeCl_3{\cdot}6H_2O$ and 0.125 resazurin. The laboratory handling of rumen fluid was carried out under a continuous flow of CO_2.

Table 1. Diet formulation and chemical composition of feed provided to the fistulated goats

Item	% (as fed)
Ingredient	
maize	22.6
wheat bran	18.0
soyabean meal	6.0
rapeseed meal	0.3
maize stover	50.0
urea	0.8
salt	0.5
mineral and vitamin premix	1.8
Chemical composition, % DM	
CP	13.8
NDF	38.0
ADF	26.0
calcium	0.27
phosphorus	0.34

CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre

About 200±10 mg of dry matter of each sample was accurately weighed into a nylon bag (12×12 mm, pore size: 45 μm). The bag prevented the feed sample adhering to the wall of the syringe. Each sample was measured in triplicate. The nylon bag containing the 200 mg samples was put into a 100 ml glass syringe fitted with a plunger. The syringes were placed in a shaking water bath (DSHZ-300, Taicang, Jiangsu, China) with 50 movements per min at 39°C, and filled with 30 ml of incubation medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution. 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 a feed sample. Gas production readings were taken immediately and after 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36 and 48 h of incubation.

Mathematical and statistical analyses

To describe the dynamics of *in vitro* gas production over time, the following Gompertz function (Schofield et al., 1994) was used:

$$GP = A \times \exp\{-\exp[1 + \frac{b \times e}{A}(LAG - t)]\}$$

where GP is cumulative gas production (ml) at different incubation times, A is the theoretical maximum gas production, b is the rate constant of gas production (ml/h), LAG is the lag time (h) which is defined as the time-axis intercept of a tangent line at the point of inflection, and t is time (h) of *in vitro* incubation. The parameters A, b and LAG were estimated by the nonlinear regression analysis procedure of SAS (1985).

The results were analysed by ANOVA using the generalized linear model procedure (SAS, 1985) with the botanical fraction as the treatment factor according to the following statistical model:

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

where Y_{ij} is a dependent variable, μ is the overall mean, T_i is a botanical fraction, and e_{ij} is the residual error. Differences among means were tested using Duncan's multiple range tests at $P<0.05$. The Pearson correlation coefficients between the chemical composition and *in vitro* gas production characteristics were determined using the Proc Corr procedure of SAS (1985). P-values < 0.05 were used to determine whether relationships were significant.

RESULTS

Chemical composition of different fractions of maize stover

The composition of different botanical fractions of maize stover used for the *in vitro* gas production measurements is presented in Table 2. The highest CP

Table 2. Chemical composition of different botanical fractions of maize stover

Botanical fraction	DM,	OM	CP	NDF	ADF	HC	NDS
	g/kg	g/kg, DM					
Whole maize stover	929	935	122	581	297	284	419
Leaf blade	928	879	162	600	324	277	400
Leaf sheath	942	911	70	697	422	275	303
Whole stem	943	928	77	601	382	220	399
Upper stem	965	945	64	658	421	238	342
Lower stem	944	934	87	575	369	206	425
Maize cob	875	934	138	591	263	328	409
Husk	944	967	43	758	384	374	242
Tassel	917	922	98	683	389	295	317

DM, dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; HC - hemicellulose; NDS - neutral detergent soluble

content in maize stover samples was in the leaf blade (16.2%), whereas the husk had the highest NDF (75.8%). On the other hand, the lowest CP and NDF were observed in the husk (4.3%) and the lower stem (57.5%). The ADF content of the maize cob (26.3%) was the lowest, while the leaf sheath and upper stem, the highest, at 42.2 and 42.1%, respectively. When comparing the chemical composition of stems, it is obvious that the lower stem had more protein and less fibre. The NDF content in the lower stem was 8% lower than in the upper stem, and CP was 2% higher in the lower than in the upper stem. The CP content of maize cob was similar to that in whole maize stover and nearly twice that found in the stem.

In vitro gas production characteristics of different botanical fractions

The *in vitro* cumulative gas production curves of different botanical fractions of maize stover are shown in Figure 1 and the parameters of the Gompertz function are listed in Table 3.

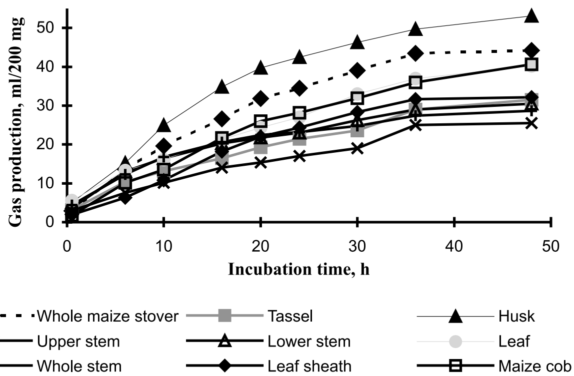

Figure 1 *In vitro* cumulative gas production of different botanical fractions

Table 3. Mean values (n=3) of *in vitro* cumulative gas produced at different times of incubation for different botanical fractions of maize stover and parameters of gas production estimated by the Gompertz function

Botanical fraction	Cumulative gas produced (ml) at h				Parameters of Gomperts function		
	12	24	36	48	A	B	Lag
Whole maize stover	22.8	34.5	43.5	44.2	45.2[ab] (3.6)	1.578[b] (0.15)	-2.2[bc] (0.5)
Lear blade	17.6	27.0	37.0	40.2	46.3[ab] (3.1)	0.980[cde] (0.13)	-6.1[f] (0.4)
Lear sheath	14.8	24.3	31.6	32.1	33.7[cd] (3.6)	1.313[bc] (0.15)	1.3[a] (0.5)
Whole stem	18.7	23.2	27.4	28.6	27.7[d] (2.8)	1.309[bc] (0.12)	-3.7[cd] (0.4)
Upper stem	11.1	17.1	25.0	25.5	29.6[d] (3.6)	0.623[e] (0.15)	-5.7[ef] (0.5)
Lower stem	18.3	23.0	29.0	30.5	29.2[d] (4.4)	1.156[bcd] (0.19)	-4.2[de] (0.6)
Maize cob	18.5	28.1	35.9	40.6	41.9[bc] (3.6)	1.194[bcd] (0.15)	-2.1[bc] (0.5)
Husk	29.1	42.5	49.7	53.0	52.9[a] (3.1)	2.182[a] (0.13)	-0.9[b] (0.4)
Tassel	14.2	21.5	29.0	31.5	34.6[cd] (2.8)	0.794[de] (0.12)	-5.4[ef] (0.4)
SEM	1.75	2.60	2.78	2.88			
Significance					0.0001	0.0001	0.0001

[a, b, c, d, e, f] means with different superscripts in the same column are significantly different ($P<0.05$). Values are estimates with their SE in parentheses. A , theoretical maximum of gas production (ml); b, rate constant of gas production (ml/h); Lag, lag time (h)

There were differences in *in vitro* gas production dynamic parameters, i.e. the theoretical maximum gas production, the rate constant of gas production and lag time among botanical fractions (see Table 3). Figure 1 clearly demonstrates the significant difference in accumulative gas production among botanical fractions of maize stover. Husk produced more gas than other fractions over a 48 h incubation period. The upper stem produced less gas than the lower stem. When all data were fitted into the Gompertz function and the parameters were calculated, husk had a higher theoretical maximum volume of gas (A) than maize cob (P<0.05), tassel (P<0.01), leaf sheath (P<0.01), upper stem (P<0.001), lower stem (P<0.001), and whole stem (P<0.001). There were no significant differences (P>0.05) between husk, leaf blade and whole maize stover in the theoretical maximum volume of gas produced. The leaf blade, whole maize stover and the maize cob had similar theoretical maximum volumes of gas. The rate constant of *in vitro* gas production was also higher (P<0.05) for husk than other botanical fractions. The rate constant of gas production was significantly lower (P<0.05) for leaf blade, tassel and upper stem than for the husk and whole maize stover, but no difference (P>0.05) was found among the whole maize stover, leaf sheath, whole stem, lower stem and maize cob. Leaf blade, upper stem and tassel had a lower lag time, but leaf sheath had the highest lag time. Maize cob had a similar lag time as whole maize stover (-2.1 vs -2.2).

Relationships between the Gompertz function parameters and chemical composition

The relationships between the Gompertz function parameters, gas volumes at 12, 24, 36, 48 h incubation and chemical composition of different botanical fractions are given in Table 4. Generally, gas volume and fermentation parameters described in the Gompertz function tended to have positive correlations with organic matter, neutral detergent fibre content, although these

Table 4. The Pearson correlation coefficients (n=9) between the parameters of Gomperts function, cumulative gas volume (ml/200mg DM) at 12, 24 36 and 48 h incubation times and chemical composition

Chemical	Cumulative gas (ml/200mg DM) at h				Parameters of Gompertz function		
composition	12	24	36	48	A	b	L
OM	0.45	0.38	0.28	0.25	0.09	0.48	0.28
CP	-0.12	-0.04	0.08	0.15	0.28	-0.32	-0.35
NDF	0.19	0.30	0.28	0.26	0.27	0.34	0.38
ADF	-0.34	-0.38	-0.43	-0.50	-0.50	-0.15	0.03
HC	0.58	0.75*	0.78**	0.83**	0.84**	0.57	0.42
NDS	-0.19	-0.30	-0.28	-0.26	-0.27	-0.34	-0.38

* levels of significance are as follows: * P<0.05; ** P<0.01; A , theoretical maximum of gas production (ml); b, rate constant of gas production (ml/h); L, lag time (h)

relationships were not significant. Acid detergent fibre and neutral detergent soluble content showed weak and negative correlations ($P>0.05$) with gas volume and fermentation parameters. There was a strong and positive correlation between hemicellulose and gas volume at 24 ($P<0.05$), 36 ($P<0.01$) or 48 h ($P<0.01$). The theoretical maximum gas production was affected by hemicellulose content ($P<0.01$).

DISCUSSION

Variation in chemical composition of botanical fractions of maize stover

In the present study, the CP content in the leaf blade was higher (16.2%) than in other fractions. This is in agreement with results found for other plant materials (e.g., grass and straw). For example in wheat straw, Tan et al. (1995) found that NDF and ADF contents were highest in the stem and lowest in the leaf blade, while the CP and NDS contents were lowest in the stem and highest in the leaf blade. In the same study they also reported that stems had a low HC but the leaf sheath had a high HC content. In sorghum stover, Sileshi et al. (1996) reported that the CP content in the leaf blade was highest and lowest in the leaf sheath. Mgheni et al. (2001) found that the nitrogen content of botanical fractions increased in the following order: stem<leaf sheath<leaf blade for rice straw and maize stover. The CP contents in the leaf blade, whole stover and even in tassel were, however, considerably higher than those found in a previous study in maize stover harvested at different stages of grain maturity (Tolera and Sundstøl, 1999). The variation is probably due to differences in varieties. The maize variety (Kexiang No.11) for this study had a high lysine content; the CP and lysine contents in the grain were up to 12.2 and 4.08% higher, respectively (HPACC, 2004). The results of our study revealed that the CP content was lowest and the NDF was highest in husk, which is consistent with the findings of Tolera and Sundstøl (1999). Verbic et al. (1995) also found a higher NDF content in husk than in the leaf blade and in the stem of the maize stover.

On the other hand, the current study also indicates that the chemical composition of the leaf sheath is similar to that of the upper stem. The most interesting finding is that the upper stem had relatively lower protein and higher fibre contents than the lower stem. This is probably due to the larger percentage of cuticle in the upper stem. Tan et al. (1995) reported there were the fluctuations in the CP and NDF contents in the upper and lower stem fractions of wheat straw. The CP content of the second stem segment was lower than that of the third stem segment, and the second stem segment contained more NDF and ADF than the third stem segment. Tan et al. (1995) also found higher CP and lower fibre contents in cob than in tassel. This is probably due to the cob sampling, because the nutrients in maize cob had not completely transferred into grain at the sampling time. These findings contradict the established facts for tropical forages (Van Soest, 1994). Further study is needed to fully understand the basic principles behind them.

Variation between botanical fractions in cumulative gas production and dynamic parameters

For the mature maize stover, there were significant variations among the different botanical fractions in *in vitro* cumulative gas production, theoretical maximum gas production, rate constant of gas production and lag time. Gas production was highest in the husk and was more rapid between 6 and 20 h of incubation. This is consistent with the findings of Tolera and Sundstøl (1999). Similarly, the results of the current study revealed that the maximum theoretical gas production and rate constant of husk was higher than other of botanical fractions. Tolera and Sundstøl (1999) also showed that the potential gas production and rate of gas production were higher in husk than in the other morphological fractions of maize stover. Tuah et al. (1996) reported that cumulative gas production for 48 h decreased in the following order: leaf blade>whole stover>stem>tassel; the rate constant of gas production decreased from leaf blade>stem>whole stover>tassel. However, the lag time decreased as follows: leaf blade>whole stover>tassel>stem. Tuah et al. (1996) also found that the *in vitro* dynamic parameters of gas production could be affected by different varieties. The present results show the cumulative gas production after 48 h of incubation for the whole stover, leaf blade, and stem were similar to the results reported by Tuah et al. (1996) except for the tassel. These differences may have resulted from using different mathematical fitting functions, from different maize varieties, or from their growing environment. Furthermore, most of the lag time values were negative. Krishnamoorthy et al. (1991b) also observed negative values of lag time for *in vitro* gas curves of oat, rye, and hay standards incubated for 96 h. They assumed that the negative lag time values are a consequence of rapid gas production in the early stages of fermentation, which may have been caused by the characteristics of the fermentable substrate.

Relationship between in vitro dynamic parameters and chemical composition

The current study has demonstrated that there were significant correlations between HC content and *in vitro* cumulative gas production, and the theoretical maximum of gas production, but other chemical composition characteristics were not related to gas volume and dynamic parameters. In the literature, the research outcomes are controversial. Khazaal et al. (1993) noted that the cumulative gas production in 12, 24, 48, 72 and 96 h incubation periods and maximum gas production were positively related to HC and CP contents, and negatively related to the ADF and NDF contents in browse. In contrast, a negative relationship was observed between gas production and CP content of morphological fractions (Tolera and Sundstøl, 1999). Cone and van Gelder (1999) also showed that the fermentation of protein causes less gas production than carbohydrate. Nsahlai et al. (1994) reported that cumulative gas production was significantly and negatively related to the ADF, NDF and HC concentrations in browses. Many factors can contribute to such conflicting outcomes. First, the raw materials used for the *in vitro* trial may be different. Raw materials such as forage and maize stover may have differences in cell wall structure and other chemical and physical characteristics that contribute to different rates of fermentation. Second, different donors of rumen fluid are used. If goat, sheep or cattle rumen fluids differ in their composition of rumen microorganisms, the substrate supplied by the raw materials will not necessarily result in the same extent of fermentation when incubated in these liquids.

CONCLUSIONS

The outcomes of this study demonstrated significant differences in chemical composition and fermentation parameters between botanical fractions of maize stover. The morphological fractions differed in the volume of gas production in the following decreasing order; husk>whole stover>leaf blade>cob>leaf sheath>lower stem>whole stem>tassel>upper stem. The hemicellulose content was significantly and positively related to cumulative gas production and theoretical maximum gas volume. These characteristics of maize stover, one of the major feed resources for ruminants, could be associated with its feeding value. The information gathered from the current study may help nutritionists to determine the nutritional value of maize stover and utilize it in developing feeding strategies for ruminants.

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