Effects of Hybrid and Maturity on Maize Stover Ruminal Degradability in Cattle Fed Different Diets

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ABSTRACT : The effect of maize hybrid (Suco and Dekalb 765, DK 765), maturity stage (milk, R_3 and 1/2 milk line, R_5) and animal diet (Diet 1: 70% lucerne hay+30% maize silage; Diet 2: 50% maize silage+20% sunflower meal+30% maize grain) on ruminal stover dry matter (DM) degradability was studied. Additionally, morphological and chemical plant composition was evaluated. Fodder samples ground at 2 mm were incubated in three Holstein steers (400 kg body weight) using the *in situ* technique. Ruminal degradation kinetics was studied and the effective degradability (ED) was estimated for an assumed kp of 5%/h. The *in situ* data was analyzed in a complete randomized block design with the animals as blocks. Significant interactions between hybrid×diet and maturity×diet on kinetic digestion parameters were detected. In Diet 1, hybrids did not differ in degradable fraction, kd or ED, although a minor difference ($p<0.05$) in the soluble fraction was found (25.5 and 23.2% for Suco and DK 765, respectively). In Diet 2, the DK 765 had greater degradable fraction ($p<0.001$) but smaller ($p<0.01$) kd than Suco, without differences in the soluble fraction or in ED. Anticipating the harvest increased ED of stover from 37.5% in R_5 to 44.6% in R_3 (average values across hybrids and diets) due to the increase ($p<0.001$) in the soluble fraction (R_5: 22.6%, R_3: 28.8%). It is concluded that hybrids had similar stover *in situ* DM degradability and that soluble fraction represent the main proportion of degradable substrates. Advancing the date of harvesting may not improve the *in situ* DM degradability of whole maize plant silage since the increase in stover quality is counteracted by the depression in the grain-to-stover ratio. The diet of the animal consuming silage might not improve stover utilization either. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1619-1624)***

Key Words : Maize Silage, Stage of Maturity, Hybrids, *In situ* Degradability

INTRODUCTION

From a nutritional point of view, whole plant corn silage is a mixture of fodder and grain, being both components of different nutritional value. It is well known that the starch supplied by grains is highly digested in the gastrointestinal tract, providing a high and rapidly available source of energy to the animal (Andrae et al., 2001). However, the fodder, or stover, might be of limited nutritional value since the complex structure of the leaf and stem cell walls present physical barriers for microbial attack (Buxton and Redfearn, 1997). Consequently, silage nutritional quality, defined in terms of degradable substrate or energy concentration, will depend on grain content and stover degradability in the rumen.

Information about factors affecting stover ruminal degradability is scarce since most research programs on maize are aimed to study factors that affect grain production. The effects of hybrids and crop management on stover nutritional quality became of particular interest in areas where the harvest index could be impaired by climate or soil conditions. In addition, it is also important to know how diets may affect stover degradation in the rumen when maize silage is used with other feeds in the diet. All these information will allow a better understanding of maize silage digestion and also is required to enhance silage nutritional value when it is used as part of a formulated diet. This research was carried out with the objective of determining the effects of hybrid, maturity stage and animal diet on *in situ* dry matter (DM) ruminal digestion kinetics of maize stover.

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MATERIAL AND METHODS

Experimental site

The experiment was carried out in Balcarce, Buenos Aires Province, Argentina (37° 45' S, 58° 18' W, 130 m above sea level). The area has an average (1991-2001) annual rainfall of 927 mm and average annual maximum and minimum temperatures of 19.8°C and 8.7°C, respectively (data from Balcarce Experimental Station).

Crop management and experimental design

Two maize (*Zea mays* L.) hybrids of 126 days of relative maturity (Suco®, from Novartis Seed Company, and Dekalb (DK) 765®, from Dekalb Genetics Corporation) were sown on 14 October 1999 in 5 row plots (10 m long and 0.7 m apart) at approximately 55,000 plants/ha. A randomized-complete block design with three replications was used. Plots were fertilized with diammonium phosphate (60 kg/ha) at sowing date and with urea (200 kg/ha) at maturity stage V_6 (Ritchie et al., 1996). At milk (21 February) and half milk line (10 March) stages, R_3 and R_5

Table 1. Effect of hybrid and maturity on maize plant morphological components (g DM per plant), ratio leaf:stem and harvest index

Item	Hybrid (H) and maturity (M)				SE	Significance		
	Suco		DK 765					
	R_3	R_5	R_3	R_5		H	M	HxM
Leaf	75.3	73.0	83.2	77.4	4.13	NS	NS	NS
Stem	80.3	86.0	84.1	98.2	5.93	NS	NS	NS
Husk	37.9[c]	27.0[c]	66.4[a]	51.0[b]	2.79	***	***	NS
Cob	28.0[b]	23.8[b]	53.1[a]	50.1[a]	3.24	***	NS	NS
Grain	36.9[c]	148.1[b]	83.7[c]	238.2[a]	14.72	***	***	NS
Leaf:stem ratio	0.95[a]	0.85[b]	1.00[a]	0.79[b]	0.03	NS	***	NS
Harvest index (%)[2]	15.6[b]	41.6[a]	22.8[b]	46.0[a]	1.91	**	***	NS

[1] Values are the means of three blocks, n=6. [2] (Grain yield/whole plant yield)×100.

[a, b] Different superscripts on means in the same row show significant (p>0.05) differences.

NS, not significant; p≥ 0.05; * p<0.05; ** p< 0.01; *** p<0.001.

respectively (Ritchie et al., 1996). two plants per plot (n=6) were randomly hand harvested (15 cm above ground). Plants were separated into stem, leaf (blade+sheath), husk, cob and grain and oven-dried at 60°C for 48 h. After weighting, stems and leaves corresponding to each hybrid and maturity stage were bulked into four compound samples (two hybrids and two maturity stages) and ground through 2 mm screen for *in situ* determination. Sub-samples were further ground to pass through a 1 mm screen and analyzed for *in vitro* DM digestibility (IVDMD, Tilley and Terry, 1963), neutral detergent fiber (NDF, Van Soest et al., 1991), crude protein (CP, N×6.25) and water soluble carbohydrates (WSC, Pichard and Alcalde, 1990). In addition, grain samples of each hybrid and maturity stage were ground through a 1 mm screen for starch determination (Mac Rae and Armstrong, 1968).

In situ ruminal DM degradability

Each ground sample (2 mm) was incubated in three Holstein steers (400±30 kg body weight), fitted with permanent ruminal canulae, in a complete randomized block design with animals as blocks. Animals, kept on individual pens with fresh water always available, were fed two diets at maintenance of body weight (AFRC, 1993). Diet 1 was formulated with 70% lucerne hay+30% whole plant maize silage and Diet 2 with 50% whole plant maize silage+20% sunflower meal+30% maize grain. Feed intake was 5.1 and 4.3 kg DM in Diet 1 and Diet 2, respectively. Rations were offered twice daily, 35% in the morning (07:30 h) and 65% in the evening (17:30 h). Animals were adapted to diets for 10 days and then *in situ* measurements were performed for an incubation period of 4 additional days. A sample of each diet components was dried (60°C) and ground (1 mm) for DM and organic matter (OM) (AOAC, 1980), IVDMD, CP, NDF, starch and WSC determination.

Ruminal fluids samples were hand taken on the first two days of incubation at 0, 4 and 8 h after the morning meal and strained through four layers of cheesecloth. A portable combination electrode pH meter (Corning Model 313, Horseheads, NY) was used to determine pH immediately after the sampling.

Two samples of 5 g DM, placed in dacron bags (10×20 cm and 50 μm mean pore size), were incubated *in situ* for periods of 4, 9, 15, 24, 48, 72 and 96 h to determine DM degradability (McDonald, 1981). The soluble fraction was determined by an *in situ* incubation of 5 minutes. All bags were soaked (39°C for 15 min) before incubation and, after removal, they were hand washed with tap water until it run clear and were then dried at 60°C for 48 h. Data from each set of sixteen bags (two per incubation period) from a single animal were fitted to the exponential equation of Ørskov and McDonald (1979): $p=a+b(1-e^{-ct})$, where p is DM degradation (%) at time t, a is the soluble fraction, b the insoluble but gradually degradable fraction and c the fractional rate of degradation. Digestion kinetic parameters were estimated by the Marquardt method with NLIN option of General Linear Model procedure of SAS (1996). The effective DM degradability was calculated at a rumen outflow rate of 5%/h (Ørskov and McDonald, 1979).

Statistical analysis

Data were analysed by the General Linear Model procedure of SAS (1996) for a complete randomised block design. The fixed effect of hybrid, maturity and their interaction were considered in the analysis of whole plant and plant component weight. The MIXED procedure of SAS (1996) was used for ruminal pH analysis using animals as blocks. For the analysis of DM degradation parameters, the fixed effects of hybrid, maturity, diet and the two and three way interactions were considered. Orthogonal contrasts to test the effects of hybrid and maturity in each diet were used. Tukey and Krammer test was used for mean difference comparisons.

RESULTS

No interaction hybrid×maturity on weights of any plant morphological component, was detected (Table 1). Leaf and

Table 2. Chemical composition of stover and grain starch content of two maize hybrids at two stages of maturity

Parameter	Hybrid and maturity			
	Suco		DK 765	
	R_3	R_5	R_3	R_5
IVDMD[1]	60.7	50.5	62.4	58.2
CP[2]	6.6	3.9	8.8	4.1
NDF[3]	51.1	56.3	53.9	58.4
WSC[4]	13.3	11.9	11.9	9.9
Starch	64.8	75.7	56.2	79.6

[1] *In vitro* dry matter digestibility. [2] Crude protein.
[3] Neutral detergent fiber.. [4] Water-soluble carbohydrates.

Table 3. Chemical composition and *in vitro* digestibility of diet components

	Lucerne hay	Sunflower meal	Whole plant maize silage	Maize grain
DM, %	88.4	92.3	29.3	90.9
	---------------------------% DM------------------------			
OM	96.0	93.5	94.6	98.9
Starch	-	-	14.3	69.7
WSC[1]	9.8	-	7.0	-
CP[2]	16.0	33.7	10.1	9.3
NDF[3]	45.3	39.4	42.5	7.2
IVDMD[4]	63.7	69.2	71.6	93.4

[1] Water-soluble carbohydrates. [2] Crude protein.
[3] Neutral detergent fiber. [4] *In vitro* dry matter digestibility.

stem DM yield per plant was not affected by hybrid (DK 765 and Suco) or maturity (R_3 and R_5), but the leaf:stem ratio decreased with maturity (0.97 to 0.82) without differences between hybrids. Husks DM yield increased with maturity being higher in the hybrid DK 765, cob weight was not affected by maturity ($p>0.05$) being lower in Suco and grain weight was affected by maturity and hybrid. In stage R_5 the hybrid DK 765 yielded 238.2 g per plant and Suco 148.1 g per plant, but no differences were detected in stage R_3. The percentage of grain in the plant or harvest index increased with maturity, being higher in the stage R_5 as expected. No differences ($p>0.05$) were detected between hybrids at the same stage of maturity (19.2% in R_3, 43.8% in R_5) in spite of the large difference in grain production per plant. However, the effect of hybrid across maturity stages was significant ($p<0.01$).

Chemical composition of maize stover and grain starch content, of both hybrids at two maturity stages is shown in Table 2. Stover IVDMD, CP and WSC decreased with the advance of maturity, NDF increased in both hybrids and grain starch content increased (60.5 to 77.6 in R_3 and R_5, respectively).

The hybrid Dekalb tended to have higher IVDMD than Suco but also higher NDF and lower WSC. The starch content tended to be higher in the hybrid Suco in R_3 but not in R_5.

The *in situ* DM degradability study was carried out in animals fed two contrasting diets. The first one was a high fiber diet, composed by 70% of lucerne hay to promote the growth of cellulolytic microorganism and to enhance fiber digestion (Hoover, 1986). The other diet was composed by 50% of whole plant maize silage, supplemented with a mix of maize grain (30%) and sunflower meal (20%) that should promote the increase of amylolytic microorganism as well as the cellulolytic microbial population. Chemical composition of diet components is presented in Table 3. Lucerne hay was of high CP, low NDF and high IVDMD and the whole plant maize silage had low NDF, high IVDMD but low starch content.

Table 4. Mean ruminal pH values of steers fed Diet 1 and Diet 2

Treatment	pH
Diet 1	6.86[a]
Diet 2	6.55[b]
SE	0.06

[a, b] Different superscripts on means show significant ($p>0.05$) differences.

Table 5. Effect of diet, hybrid and maturity on kinetic parameters of DM degradation of maize stover

Diet	Hybrid	Maturity	a %	b %	c %/h	ED[1] %
D1	SC	R_3	28.2	45.3	2.8	48.1
		R_5	22.7	47.1	3.1	40.3
	DK	R_3	26.3	49.6	3.8	47.6
		R_5	20.1	54.2	2.9	39.6
D2	SC	R_3	30.1	46.4	2.8	41.6
		R_5	24.2	33.7	3.4	35.7
	DK	R_3	30.4	58.3	1.1	40.9
		R_5	23.2	56.5	1.2	34.3
SE			0.5	2.8	0.4	0.8
CV (%)			5.5	11.3	29.0	3.5
Maturity (M)			***	NS	NS	***
Hybrid (H)			**	***	*	NS
Diet (D)			**	NS	**	***
Block			NS	NS	NS	-
MxH			NS	NS	NS	NS
MxD			NS	*	NS	NS
HxD			*	*	**	NS
CONTRAST						
SC vs. DK D1			**	NS	NS	
SC vs. DK D2			NS	***	***	
R_3 vs. R_5 D1			***	NS	NS	
R_3 vs. R_5 D2			***	*	NS	

[1] For a kp= 5%/h. NS, not significant: $p\geq0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. SC: Suco; DK: Dekalb 765; R_3: milk; R_5: half milk line.

No diet×time interaction ($p>0.05$) for ruminal pH was found. Average ruminal pH was higher ($p<0.05$) in the diet rich in lucerne hay (Diet 1) across all sampling times (6.86 vs. 6.55 for Diet 1 and 2, respectively). This was expected since Diet 2 had 30% of maize grain (Table 4).

Degradability parameters of maize stover are shown in Table 5. The soluble fraction decreased ($p<0.001$) with maturity across hybrids and diets. There was a significant ($p<0.05$) hybrid×diet interaction, being the soluble fraction higher ($p<0.01$) in hybrid Suco than in DK 765 (25.5% vs.

23.2%) when animals were fed Diet 1. However, when fed Diet 2 no effect of hybrid was observed (27.0% in average). Significant ($p<0.05$) interactions between diet, maturity and hybrid on the insoluble but degradable fraction were detected. In Diet 1, this fraction was not affected ($p>0.05$) by maturity or hybrid (49.1% in average), but in Diet 2, this fraction was higher in DK 765 (57.4%) than in Suco (40.1%) and in stage R_3 (52.4%) than in R_5 (45.1%). However, maturity across hybrids did not affect this fraction ($p>0.05$). The fractional degradation rate was not affected by stage of maturity across hybrids and diets ($p>0.05$), but there was a significant ($p<0.05$) interaction hybrid×diet. For example, the degradation rate of Suco in Diet 2 was higher than in DK 765 (3.1%/h and 1.2%/h, respectively), but no effect of hybrid was detected in Diet 1 (average 3.2%/h). The effective DM degradability, at 5%/h rumen outflow rate, was not influenced by hybrid, but it was affected ($p<0.001$) by maturity stage and diet. It was higher in R_3 (44.6%) than in R_5 (37.5%) and in Diet 1 (43.9%) than in Diet 2 (38.1%).

DISCUSSION

Maturity and hybrid did not affect stover yield (leaf+stem yield) although Russell (1986) and Irlbeck et al. (1993) reported a decrease in stover yield with maturity. Data indicate that maturity was more important than hybrids in determining the proportion of grain in the plant and the stover morphological composition. In fact, hybrid did not affect the harvest index nor the ratio leaf to stem. It is important to note that the hybrid Dekalb, harvested in stage R_5, yielded 60.8% more grain per plant than Suco, but both hybrids have similar percentage of grain, leaf and stem in the plant. In other words, DK 765 yielded more grain per plant because plant were heavier but not of higher grain proportion.

The increase of grain proportion in the plant (19.2% in R_3 to 43.8% in R_5 across hybrids) with the concomitant decrease in the stover fraction was the most important effect of maturity, which is in agreement with data of Russell (1986); Hunt et al. (1989); Tolera et al. (1998) and Kim et al. (2001). In addition, the ratio leaf to stem decreased from 0.98 in R_3 to 0.82 in R_5 due to an increased in stem yield and leaves losses with maturity (Tolera et al., 1998) as shown in Table 1.

Stover *in vitro* digestibility, crude protein and water-soluble carbohydrates decreased from R_3 to R_5 and neutral detergent fiber increased as expected (Flachowsky et al., 1993; Tolera et al., 1998; Firdous and Gilani, 1999). Changes in stover composition were in parallel with the increase in grain starch content (Hunt et al., 1989; Russell, 1996; Tolera et al., 1998). If the increase in grain content counteracts the decrease in stover quality, the whole plant *in vivo* digestibility does not change with maturity (Bal et al., 1997; Johnson et al., 1999; Di Marco et al., 2002).

Grain addition to the diet decreased average ruminal pH ($p<0.05$), which has been associated with fiber digestibility depression (Hoover, 1986; De Visser et al., 1998; Heldt et al., 1998; Bodine et al., 2001). Nonetheless, Hoover (1986) reported that only when pH reach values closer or below 6 fiber digestibility would be affected. In our study, ruminal pH were always above 6.3, which suggest that cellulolytic bacteria growth might be not impaired. However, the stover DM fractional rate of degradation was higher in Diet 1 than in Diet 2 (4%/h and 2.2%/h, respectively), in spite that both diets showed high pH values (6.86 vs. 6.55, respectively). Canton and Dhuyvetter (1997) suggested that ruminal pH may not explain per se the depression in fiber digestibility, since others factors, such as essential nutrients or microorganisms preference for substrates, might be important in fiber digestion.

Ruminal kinetic parameters presented interactions diet x hybrid and diet x maturity as showed previously (Table 5).

The soluble fraction was affected by hybrid only in Diet 1 ($p<0.05$), but such difference may not be of practical importance since it was of 2 units percent. As maturity progressed from R_3 to R_5, this fraction decreased from 28.8 to 22.6%, across all other factors. This is consistent with Tolera et al. (1998), although they reported lower values, probably because they used crops in more advanced stages of maturity. Decrease in the soluble fraction with maturity could be attributed to translocation of cell-soluble substances to grains, which might occur without any change in the whole plant quality (Daynard and Hunter, 1975). Important is to note that although re-mobilization of soluble carbohydrates affects stover quality, it may be beneficial for silage quality. In other words, soluble carbohydrates are stored as starch in grains avoiding losses during ensiling and consequently assuring more energy available for the ruminant (Wilkinson and Phipps, 1979; Ballard et al., 2001).

The insoluble but degradable fraction was not affected ($p>0.05$) by hybrids and stages of maturity in Diet 1, being in average 49.1%. However, both factors affected this parameter in Diet 2, being higher in hybrid DK 765 (57.4%) than in Suco (40.1%) and in stage R_3 (52.4%) than in R_5 (45.1%). Anyway, ED differences between hybrids were canceled out because the hybrid with higher insoluble but degradable fraction had a smaller rate of degradability. The average ED across hybrids was 41.3% in stage R_3 and 35.0% in stage R_5.

From a nutritional point of view, the degradable substrate provided by the stover will depend on the contribution of two different pools. One is the soluble fraction content, which depends on the amount of soluble carbohydrates stored in stover less the fraction lost by respiration and drip off during ensiling and storage. The second pool is the insoluble but degradable fraction degraded by ruminal microorganisms during the time that silage is retained in rumen. According to our data both pools contributed in equal amount to the degradation of stover in 24 h. Arieli et al. (1998) also reported that the soluble fraction explained half of the stover 24 h disappearance. Evaluation of contribution of structural components and soluble carbohydrates in stover, as energy for ruminants, will allows a better understanding of silage digestion.

IMPLICATIONS

The hybrid recommended for silage (Suco) and the hybrid used for grain production (Dekalb 765) presented no differences in harvest index when compared at the same stage of maturity, although the hybrid DK 765 yielded 60.8% more grain per plant. As maturity progressed, stover quality decreased and the harvest index increased. The stover fraction of both hybrids did not differ in effective degradability (41.0% in average across all other factors) and both were poorly degraded in the rumen in both diets. Half of the ED was explained by the soluble fraction, which decreased with the advance of maturity. However, harvesting in earlier stages to increase the soluble fraction might not improve whole plant silage *in situ* DM degradability since such increase is counteracted by the depression in the ratio grain:stover.

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