Effects of chop length and ensiling period of forage maize on *in vitro* rumen fermentation characteristics

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Abstract

The effect of chop length and ensiling period on *in vitro* rumen fermentation characteristics of forage maize was studied in two experiments. In the first experiment, maize plants of eight cultivars representing different combinations of Dry Down, Stay Green, early ripening, late ripening, starch and cell wall types were chopped at harvest into pieces of 6 or 15 mm and ensiled in small laboratory silos. After 8 weeks, silage samples were taken and freeze-dried (not ground) before *in vitro* rumen fermentation characteristics were determined using the gas production technique. Chop length appeared not to affect the *in vitro* fermentation characteristics. In the second experiment, plants of two of these maize cultivars were chopped into 6-mm pieces and ensiled for different periods in small laboratory silos and in large bunker silos as used in practice. After 0, 14, 42 and 180 days of ensiling, chemical composition and *in vitro* rumen fermentation characteristics were determined using the gas production technique. The *in vitro* fermentation characteristics were not influenced by the ensiling period up to 180 days. During the first two weeks of the ensiling period sugar content decreased and so did the gas production caused by fermentation of the soluble components.

Additional keywords: degradability, gas production technique, harvest date, maize type

Introduction

In Western Europe, silage maize – next to grass – is the major component in rations for dairy herds. In the past decades much research has been done to establish the optimal ensiling conditions, such as harvest date (Mayombo *et al.*, 1993; Hartmann *et*

al., 2000), dry matter content of the plants at harvest (Yahaya *et al.*, 2002), genotype (Schwarz *et al.*, 1996; Argillier & Barrière, 1996; Johnson *et al.*, 2003), weather conditions during growth (Meisser & Wyss, 1998), breeding strategies (Barrière *et al.*, 1997; Bavec & Bavec, 2002), and physical properties of the ensiled material (Stockdale & Beavis, 1994; Johnson *et al.*, 2003). To increase the rate of ensiling fermentation and to achieve a quick drop in pH of the silage, additives, such as molasses, organic acids (Jaakkola *et al.*, 2006) and bacterial preparations are used. Bacterial preparations are also used to enhance aerobic stability after opening the silo (Weinberg *et al.*, 2002; Muck, 2004). These factors influence the ensiling process, which in turn can influence the feeding quality of the silage through differences in rumen fermentation behaviour. Since fully automated equipment is available, fermentation characteristics in rumen fluid can easily be determined *in vitro* with the gas production technique (Cone *et al.*, 1996). Although many investigations have been described that studied the influence of different ensiling factors on rumen fermentation characteristics, nearly all these studies were performed using silage after a fixed period of ensiling. Only a few studies describe the influence of the length of the ensiling period on the quality of the silage (Lee *et al.*, 2002; Yahaya *et al.*, 2002).

The aim of this study was to determine the influence of chop length (6 mm vs. 15 mm) and length of the ensiling period, up to 180 days, on *in vitro* rumen fermentation characteristics, using the gas production technique (Cone *et al.*, 1996).

Materials and methods

Maize samples

Eight maize genotypes were sown on 9 May 2003 on a clay soil in Lelystad, The Netherlands, on plots of 8.5 m × 9 m, at a rate of 95,000 seeds ha^{-1}. The genotypes represented different combinations of Dry Down, Stay Green, early ripening, late ripening, predominantly cell wall or starch type traits. Cultivar (cv.) 1 is a Dry Down, early ripening predominantly starch type; cv. 2 is a Dry Down, early-ripening cell wall type; cv. 3 is a Stay Green, early-ripening starch type; cv. 4 is a Stay Green, early-ripening cell wall type; cv. 5 is a Dry Down, late-ripening starch type; cv. 6 is a Dry Down, late-ripening cell wall type; cv 7. is a Stay Green, late-ripening starch type, and cv. 8 is a Stay Green, late-ripening cell wall type. The maize was fertilized at a rate of 175 kg N and 215 kg P2O5 ha^{-1} $year^{-1}$; the crop was not irrigated. The cultivars were allocated randomly to the plots. Each plot consisted of 12 plant rows. The middle 2 rows were meant for chemical analysis and *in vitro* research (Cone *et al.*, 2008); the adjacent 6 rows (3 on either side) were mechanically harvested and meant for experiments with ensiled maize. Of each row only the central 4 m were used for observations. On each harvest date one plot of each genotype was harvested. Plants harvested for ensiling were mechanically cut into pieces of 6 or 15 mm. Samples for chemical analysis were dried at 70 °C and ground over a 1-mm sieve. The effect of chop length was investigated for all 8 genotypes.

The harvested and cut material was ensiled for different periods in large bunker silos normally used in practice, and in small 18-litre laboratory silos with the possibility

to drain the pressure juice. Each laboratory silo was filled with 10 to 15 kg cut material and was pressurized with a block of 30 kg to simulate a silo height of 2 m. To compare silages cut at 6 mm with silages cut at 15 mm, the silages were stored for 8 weeks at 20 °C, opened and freeze-dried. The silage samples that were used to determine the fermentation characteristics were not ground. The samples for chemical analysis were dried at 70 °C and ground over a 1-mm sieve. To investigate the effect of the ensiling period, silages of cultivars 2 and 3 chopped at 6 mm and harvested at two different dates were ensiled for 0, 14, 42 or 180 days before opening the silos.

Chemical analysis and digestibility

Dry matter (DM) content was determined gravimetrically by drying for 4 h at 103 °C (ISO 6496) and ash content was determined by incineration for 3 h at 550 °C (ISO 5984). Starch content was determined, after pre-extraction with ethanol (40% v/v), as glucose, using the amyloglucosidase method (Keppler & Decker, 1970) after liberating the starch by autoclaving for 2 h at 120 °C. Crude protein (CP), neutral detergent fibre (NDF) and sugars were determined by near infrared reflectance spectroscopy (NIRS) (Murray, 1993; Deaville & Flinn, 2000).

Gas production incubations

Rumen fluid was collected from two non-lactating rumen-cannulated cows 2 h after the morning feeding. The cows were being fed 1 kg of standard compound feed containing about 150 g starch per kg DM in the morning and *ad libitum* hay in the morning and the afternoon. The rumen fluid from the two cows was combined, stored in a warm insulated flask filled with CO_2, filtered through cheesecloth, and mixed (1:2 v/v) with an anaerobic buffer/mineral solution as described by Cone *et al.* (1996). Duplicate samples of 0.5 g DM were incubated in 60 ml buffered rumen fluid in 250-ml bottles in a shaking water bath at 39 °C. Gas production was recorded for 48 h using a fully automated system (Cone *et al.*, 1996). All manipulations were done under continuous flushing with CO_2. Gas productions were corrected for blank gas productions (i.e., gas productions in buffered rumen fluid without sample).

Gas production curves were modelled as described by Cone *et al.* (1996) and Groot *et al.* (1996). A gas production curve can be divided into three sub-curves, each of which with an asymptote (A), a half-time value (B) and a shape parameter (C) (Groot *et al.*, 1996). The asymptotes of sub-curve 1 (A1) and 2 (A2) and the half-time value of sub-curve 2 (B2) are most important for feed evaluation. A1 corresponds to the gas production caused by fermentation of the water soluble components and A2 corresponds to the gas production caused by fermentation of the non-soluble components (Cone *et al.*, 1997), which mainly consist of cell walls and starch. The half-time value B2 is the incubation period (h) needed to reach half of A2 and is a measure for the rate of cell wall and starch degradation.

Starch degradation after 10 h of incubation in rumen fluid using the gas production technique was calculated using the amount of gas produced after 10 h and the starch content in the not fermented sample (Chai *et al.*, 2004).

Experimental design and statistical analysis

Single plots of the different forage maize types were mechanically harvested, cutting the forage into 6- or 15-mm pieces. Of the harvested forage a sample of 20 kg material was taken at random for chemical analysis, gas production incubations and laboratory ensiling. The remaining forage was ensiled in large bunker silos. From the not ensiled material as well as from the ensiled material duplicate samples were taken for analysis. Variance sources (main effects and interactions) were analysed with an ANOVA (Analysis of Variance) procedure, resulting in F-probabilities of the variance sources. Based on the results of the ANOVA procedure, least significant differences (LSD) were determined with a Student t-test ($P < 0.05$). All calculations were performed in Statistix 8.0 (Statistix Analytical Software, Tallahassee, Florida, USA).

Results

Chop length

Starch content and the *in vitro* rumen fermentation parameters obtained with the gas production technique for the silages of the eight cultivars are summarized in Table 1. The table also gives the calculated amount of starch degraded after 10 h of incubation *in vitro* in rumen fluid. The results show that there were no statistically significant effects of chop length on gas production characteristics. Also the differences in the calculated amount of degraded starch (Chai *et al.*, 2004) were not statistically significant. Statistically significant ($P < 0.05$) differences between cultivars were only observed for A1 and for the calculated amount of degraded starch. The effect for A1 was mainly caused by the relatively high value for cv. 6, compared with the other cultivars. The interaction chop length × cultivar was only statistically significant ($P < 0.05$) for B2.

In vitro rumen fermentation characteristics

Table 2 gives an overview of the chemical composition of the samples of the maize cultivars 2 and 3, harvested on 2 and 25 September and ensiled for different periods. The results show that the dry matter content was highest for the samples of 2 September and that starch content was highest for the 25 September samples. Concomitantly the NDF content was lower in the more mature samples. Sugar content was highest in the not ensiled (0 days) samples and stayed rather low during the ensiling period.

Table 3 presents the obtained gas production parameters of the samples investigated, and Table 4 the effects of the different treatments on the gas production parameters. The results show that the ensiling period had no influence on the fermentation of the non-soluble components (starch and cell walls) (A2) or on the rate of it (B2), but that it did affect the fermentation of the soluble components (A1). The gas production parameters were not significantly ($P < 0.05$) influenced by the type of silo used, but the effect of harvest date was statistically significant ($P < 0.05$). The fermentation of the soluble components was significantly different between

Table 1. Starch content, degraded starch content and gas production parameters of maize silage of 8 cultivars. Maize plants were chopped at 6 or 15 mm and ensiled for 8 weeks in small laboratory containers. The plants were harvested at a dry matter content of about 320 g per kg.

Chop length	Cultivar	Starch content		Gas production parameters [1]				Degraded starch content
				GP 20	A1	A2	B2	
(mm)		(g per kg DM)		- - - - - - -	(ml per g OM)	- - - - - -	(h)	(g per kg)
6	1	355		219	23.6	195	10.30	324
	2	299		199	28.0	171	10.23	287
	3	362		193	22.6	170	10.35	257
	4	321		245	24.1	221	10.59	367
	5	329		251	27.5	223	10.16	415
	6	220		234	36.4	197	10.01	459
	7	347		249	24.3	225	10.64	359
	8	322		218	29.2	188	10.95	295
15	1	355		231	22.5	209	10.50	324
	2	299		253	28.9	224	10.73	380
	3	362		164	24.1	140	11.11	171
	4	321		196	23.7	173	10.72	227
	5	329		269	24.9	244	10.46	437
	6	220		255	37.7	217	10.35	504
	7	347		191	26.4	165	10.09	269
	8	322		239	29.5	209	10.48	373
			LSD [2]	61	3.0	60	0.57	172
Overall averages								
6 mm				226	26.9	199	10.40	345
15 mm				225	27.2	198	10.55	336
			LSD	21	1.0	21	0.20	68
	1			225	23.0	202	10.40	324
	2			226	28.5	198	10.48	335
	3			178	23.3	155	10.73	214
	4			220	23.9	197	10.65	297
	5			260	26.2	233	10.31	426
	6			244	37.0	207	10.18	482
	7			220	25.3	195	10.36	314
	8			228	29.4	199	10.71	334
			LSD	43	2.1	42	0.40	118
Statistical effects [3]								
Chop length				ns	ns	ns	ns	ns
Cultivar				#	***	#	#	**
Chop length × cultivar				ns	ns	ns	*	ns

[1] See text for explanation of parameters.

[2] LSD = least significant difference ($P < 0.05$).

[3] Statistical significance: # = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 2. Dry matter content and chemical composition of silage of maize cultivars 2 and 3 harvested on 2 September (02-09) and 25 September (25-09). Chopped maize plants (6 mm) were ensiled for 0, 14, 42 or 180 days in small laboratory or in large bunker silos.

Cultivar/ silo type	Harvest date	Ensiling period	DM content	Chemical composition				
				Ash	CP	NDF	Starch	Sugars
		(days)	(g kg^{-1})	(g per kg DM)				
Cultivar 2								
	02-09	0	319	35	63	407	292	94
	25-09		391	43	63	378	367	46
Small	02-09	14	310	40	68	384	315	8
	25-09		354	38	62	411	341	6
	02-09	42	290	40	69	402	293	8
	25-09		363	44	59	409	335	6
	02-09	180	281	44	65	437	278	7
	25-09		302	50	48	504	258	6
Large	02-09	14	328	39	57	466	261	8
	25-09		349	44	61	443	304	7
	02-09	42	237	49	78	455	282	6
	25-09		365	61	62	408	319	6
	02-09	180	312	44	77	368	324	13
	25-09		375	40	66	383	354	8
Cultivar 3								
	02-09	0	287	43	61	403	294	111
	25-09		364	56	58	400	328	56
Small	02-09	14	248	51	65	422	284	14
	25-09		354	43	64	367	383	6
	02-09	42	227	54	62	445	238	8
	25-09		315	49	62	369	366	6
	02-09	180	290	45	67	387	314	7
	25-09		369	49	63	351	392	6
Large	02-09	14	303	45	66	419	281	26
	25-09		338	47	62	372	373	6
	02-09	42	276	52	70	376	305	11
	25-09		322	47	63	403	347	6
	02-09	180	277	44	65	417	259	15
	25-09		338	46	61	390	345	7

Table 3. Gas production parameters of silage of maize cultivars 2 and 3 harvested on 2 September (02-09) and 25 September (25-09). Chopped maize plants (6 mm) were ensiled for 0, 14, 42 or 180 days in small laboratory or in large bunker silos.

Cultivar/ silo type	Harvest date	Ensiling period	Gas production parameter [1]		
			A1	A2	B2
		(days)	- - - (ml per g OM) - - -		(h)
Cultivar 2					
	02-09	0	34.8	229.6	7.45
	25-09		24.6	240.2	7.51
Small	02-09	14	27.7	232.8	7.18
	25-09		20.8	237.9	7.40
	02-09	42	34.0	227.9	7.35
	25-09		19.6	234.6	7.56
	02-09	180	25.5	213.2	7.21
	25-09		18.3	223.0	7.69
Large	02-09	14	30.2	215.9	7.50
	25-09		16.7	227.7	7.93
	02-09	42	12.8	216.2	7.80
	25-09		13.8	232.2	7.50
	02-09	180	30.9	230.3	7.30
	25-09		20.9	232.2	7.50
Cultivar 3					
	02-09	0	40.2	226.3	7.35
	25-09		27.3	228.2	7.52
Small	02-09	14	37.7	220.5	7.54
	25-09		24.0	233.0	7.42
	02-09	42	38.0	209.0	7.11
	25-09		21.8	232.1	7.46
	02-09	180	32.1	226.0	7.21
	25-09		22.7	236.5	7.05
Large	02-09	14	33.8	216.7	7.03
	25-09		21.4	235.0	7.47
	02-09	42	33.8	221.4	7.26
	25-09		20.5	228.5	7.55
	02-09	180	35.8	211.6	7.32
	25-09		24.5	231.2	7.19
LSD [2]			5.6	13.9	0.46

[1] A1 = maximum gas production caused by fermentation of the soluble fraction;
A2 = maximum gas production caused by fermentation of the non-soluble fraction;
B2 = time to reach half of A2.

[2] LSD = least significant difference ($P < 0.05$).

Table 4. Overall effects of cultivar, harvest date, type of silo, and ensiling period on the gas production parameters of silage of maize plants harvested on 2 September (02-09) and 25 September (25-09). Chopped maize plants (6 mm) were ensiled for 0, 14, 42 or 180 days in small laboratory or in large bunker silos.

	Gas production parameter [1]		
	A1	A2	B2
	- - - - - (ml per g OM) - - - - - -		(h)
Cultivar			
Cv 2	24.5 b [2]	228.9	7.48 a
Cv 3	30.5 a	226.2	7.31 b
Harvest date			
02-09	32.9 a	222.1 b	7.32 b
25-09	22.1 b	233.1 a	7.47 a
Type of silo			
Small	26.3	226.4	7.45
Large	28.6	228.7	7.35
Ensiling period (days)			
0	32.8 a	232.2	7.41
14	26.5 b	227.4	7.43
42	24.3 b	225.2	7.45
180	26.3 b	225.5	7.31
Statistical effects [3]			
Cultivar	***	ns	**
Harvest date	***	***	*
Type of silo	#	ns	ns
Ensiling period	***	ns	ns

[1] A1 = maximum gas production caused by fermentation of the soluble fraction;
A2 = maximum gas production caused by fermentation of the non-soluble fraction;
B2 = time required to reach half of A2.

[2] Means in the same column, followed by a different letter are statistically different ($P < 0.05$).

[3] Statistical significance: # = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$;
ns = not statistically significant ($P \geq 0.1$).

the cultivars 2 and 3. This was not the case for the fermentation of the non-soluble components (A2), but the rate of fermentation of the non-soluble components (B2) was significantly different between these two cultivars.

Discussion

Chop length

The results (Table 1) clearly show that there were no statistically significant ($P < 0.05$) differences in the gas production parameters between the two chop lengths investigated. This indicates that there was no statistically significant ($P < 0.05$) difference in total gas production after 20 h of incubation (GP20), in the degree of fermentation of the water soluble components (A1) or in the rate (B2) and extent (A2) of degradation of the non-soluble components (cell walls and starch). The effect of chop length on starch degradation as determined with the equation of Chai *et al.* (2004) was not statistically significant ($P < 0.05$) either. As the dried silage samples with a chop length of 6 and 15 mm were incubated in rumen fluid, using the gas production equipment, this indicates that particle size did not have a statistically significant ($P < 0.05$) influence on *in vitro* rumen fermentation characteristics.

Several authors have studied the effect of chop length. Stockdale & Beavis (1994) showed that chop length of maize silage generally appeared to have little or no effect on silage quality or milk production. Johnson *et al.* (2003) observed no effects of chop length (11–40 mm) on the *in situ* disappearance of DM, starch or CP, but showed variable effects of chop length on *in situ* NDF disappearance. Schwab *et al.* (2002) did not find effects of increasing chop length of brown-midrib maize silage on lactation performance, although an increased chop length slightly decreased the dry matter intake, as was also shown by Couderc *et al.* (2006) comparing 6-mm with 23-mm maize chops. Onetti *et al.* (2003) observed no difference in dry matter intake, milk fat production or rumen fermentation end products when feeding cows with maize silage chopped at 19 or 32 mm. Comparing barley silages of 10 and 19 mm Einarson *et al.* (2004) observed an increased dry matter intake of the total mixed ration with the small particles, but observed no effect of chop length on rumen pH, total volatile fatty acids, milk yield or milk composition.

Based on our results we conclude that chop length of the ensiled maize plants did not have an influence on *in vitro* rumen fermentation characteristics. From the literature it can be concluded that chop length also has little or no influence on *in vivo* degradation and on animal performance. Obviously the ensiling process and the long ensiling period make the particle size of minor importance. In the *in vivo* situation, rumination may level off possible differences.

In vitro rumen fermentation characteristics

The summarized results presented in Table 4 show that the gas production caused by fermentation of the soluble fraction (A1), was statistically higher ($P < 0.05$) for silages of cultivar 3 than for silages of cultivar 2. Similar differences between these two cultivars were found by Cone *et al.* (2008). The two cultivars did not differ in extent of fermentation of the non-soluble components (Cone *et al.*, 1997), and only slightly in rate of fermentation. Harvest date showed a statistically significant ($P < 0.05$) influence on all gas production parameters, as has been found earlier by Struik (1983), Cone &

Engels (1993) and Cone *et al.* (2008). There appeared no statistically significant ($P < 0.05$) difference in gas production parameters between the small laboratory silos and the large bunker silos used in practice, indicating that the small silos were a good imitation of the large ones.

The length of the ensiling period only had a statistically significant ($P < 0.05$) effect on the gas production parameter A1, indicating differences in degree of fermentation of the soluble fraction. This seems logical since the sugars in the maize (0 days ensiling) will be fermented during the ensiling period, as was also shown by Yahaya *et al.* (2002). Up to 180 days, the ensiling period did not affect the rate (B2) and extent (A2) of fermentation of the non-soluble components. Obviously, only the sugars are used during fermentation, whereas the cell walls and starch stay unaffected. Also after prolonged ensiling the feeding quality stays constant. Although this is generally assumed, hardly any evidence was found in the literature. Yahaya *et al.* (2002) observed that increasing ensiling time of high moisture orchard grass resulted in excessive losses of dry matter, water soluble components, hemicellulose and cellulose. On the other hand, Lee *et al.* (2002) did not find differences in chemical composition of total mixed rations, including maize, following ensilage.

Conclusions

The following conclusions can be drawn:

1. Chop length (6 mm vs. 15 mm) of maize plants at harvest did not influence the *in vitro* rumen fermentation characteristics determined after 8 weeks of ensiling.
2. *In vitro* fermentation characteristics determined by means of the gas production technique were not influenced by the length of the ensiling period up to 180 days. During initial ensiling (0–14 days) the plant's sugars are used for the fermentation of the silage.

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