Effects of forage maize type and maturity stage on *in vitro* rumen fermentation characteristics

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Abstract

An experiment with forage maize plants representing early and late-ripening types of Dry Down and Stay Green cultivar types was conducted to study the effects of cultivar and maturity stage on *in vitro* rumen fermentation characteristics and to investigate the validity of the generally supposed qualities of these cultivars. Plants were harvested at an estimated whole plant dry matter (DM) content of 250, 320 or 390 g kg^{-1}, on 20 August, 16 September and 3 October 2003, respectively. Chemical composition and *in vitro* rumen fermentation characteristics, using the gas production technique, were determined of samples from entire not ensiled plants, ears and stover and from entire plants after ensiling. The increase in whole plant DM content from 250 to 320 g kg^{-1} (20 August – 16 September) caused starch content of the whole plants to increase and neutral detergent fibre (NDF) digestibility to decrease, both more than prolonged ripening (to 390 g DM kg^{-1}). DM content at harvest had a statistically significant influence on degree and rate of *in vitro* rumen fermentation. Calculated *in vitro* starch degradation after 10 h of incubation in rumen fluid suggested an increased content of rumen escape starch in the older samples. Maize type had only minor effects on fermentation characteristics, which were most pronounced for the ears and the remaining stover. Although the observed differences caused by the Dry Down or Stay Green characteristics were statistically significant in some cases, they were not systematic not for the early nor for the late-ripening types.

Additional keywords: Dry Down, harvest date, maize type, starch degradability, Stay Green

Introduction

In past decades, forage maize breeding introduced a wide variety of maize genotypes

(Derieux *et al.*, 1987; Rebourg *et al.*, 2003; Barrière *et al.*, 2006) differing in earliness, ripening and kind of energy (cell walls or starch). Consequently, maize cultivars vary in plant composition (stover, ear), optimum harvest date and rumen fermentation characteristics, and in feeding value (Frei, 2000; Troyer, 2000; Duvick, 2005). Commercial maize cultivars are marketed as being early ripening, late ripening, Dry Down, Stay Green, as a starch type or as a cell wall type. The stems and leaves of the Stay Green (SG) types are supposed not to senesce rapidly during ear ripening and to remain green during the grain filling period, whereas the stems and leaves of the Dry Down (DD) types senesce faster, turning brown during the final filling of the ear. Consequently, at the same harvest date the SG types may have a higher digestibility than the DD types, although some studies (Schlagheck *et al.*, 2000) do not prove this for the whole plant whereas others only prove it for the stover (Hartmann *et al.*, 2000; Schlagheck *et al.*, 2000). In many cases the influence of maize type on rumen fermentation characteristics remains unclear. Certainly, the effect of harvest date on rate and extent of rumen fermentation of both the cell walls and the starch is not always well understood (Schwarz & Ettle, 2000). However, Cone & Engels (1993) have shown a large influence of plant maturation on cell wall chemical composition and degree of cell wall degradation in rumen fluid. Not only the energy content of the maize is important, but also its composition (starch vs. cell wall). Two maize silages with the same energy value may differ in animal performance because of a different energy composition (De Boever *et al.*, 1993; Schwarz *et al.*, 1996).

The aim of this study was to determine the influence of maize type (Stay Green, Dry Down, early ripening, late ripening) and DM content at harvest (ripening stage) on rumen fermentation characteristics. Using the gas production technique (Cone *et al.*, 1996), fermentation kinetics were determined of samples of whole plants, stover (mainly cell wall), ears (mainly starch) and of ensiled plants.

Materials and methods

Maize samples

Four forage maize genotypes were sown on 9 May 2003 on a clay soil in Lelystad, The Netherlands, on plots of 8.5 m x 9 m, at a rate of 95,000 plants ha^{-1}. The genotypes represented early-ripening and late-ripening cultivars of the Dry Down (DD) and the Stay Green (SG) types, which are supposed to have a relatively high starch content. Genotypes were allocated randomly to the plots. The maize was fertilized at a rate of 175 kg N and 215 kg P2O5 ha^{-1} $year^{-1}$; no irrigation took place. Each plot consisted of 12 plant rows, of which the central two and of these the central 4 m were used for chemical analysis and *in vitro* research. Maize plants were harvested during ripening at three different dates, chosen such that the plants had a dry matter (DM) content of approximately 250, 320 or 390 g kg^{-1}. To decide on the correct stage, plants were sampled at regular intervals, cut into small pieces and dried rapidly in a microwave oven to determine their DM content. As during ripening little difference in DM content appeared between the maize types, no distinction was made between harvest date for the various cultivars: the four types were harvested on 20 August, 16 September and 3 October 2003. On each harvest date one plot of each genotype was harvested. Part of the harvested plants were mechanically cut into 6-mm pieces and used for ensiling or for chemical analysis after drying (at 70 °C) and grinding over a 1-mm screen (hammer mill, Peppink, Olst, The Netherlands). The rest was separated by hand into ears and stover. Samples for *in vitro* gas production experiments were freeze-dried.

For ensiling, the maize plants were cut into 6-mm pieces, which were placed for 8 weeks at 20 °C in 18-litre containers (laboratory silos) with the possibility to drain the pressure juice. Each silo was filled with 10 to 15 kg cut material and was pressurized with a weight of 30 kg, simulating a silage height of 2 m. After 8 weeks, samples were taken, dried at 70 °C or freeze-dried and ground over a 1-mm screen.

Chemical analysis and digestibility

Dry matter (DM) content was determined gravimetrically by drying for 4 h at 103 °C (ISO 6496), and ash was determined by incineration for 3 h at 550 °C (ISO 5984). Starch content was determined, after a pre-extraction with ethanol (40% v/v), as glucose, using the amyloglucosidase method of Keppler & Decker (1970) after liberating the starch by autoclaving for 2 h at 120 °C. Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and organic matter digestibility (OMD) were determined by near infrared reflectance spectroscopy (NIRS; Murray, 1993; Deaville & Flinn, 2000). NDF digestibility (NDFD) was determined after 48 h of incubation in buffered rumen fluid, followed by treatment with neutral detergent solution (Goering & Van Soest, 1970).

Gas production technique

The incubation experiments were carried out with rumen fluid collected from two non-lactating rumen-cannulated cows 2 h after the morning feeding. In the morning the cows received 1 kg of standard compound feed containing about 150 g starch per kg DM, and *ad libitum* hay in the morning and in 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). Samples of 0.5 g DM were incubated in duplicate with 60 ml buffered rumen fluid in 250 ml bottles placed 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 took place under continuous flushing with CO^2. Results were corrected for blank gas production (i.e., gas production in buffered rumen fluid but without a sample).

Gas production curves were modelled as described by Cone *et al.* (1996) and Groot *et al.* (1996). The gas production curves are divided into three different sub-curves, each 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) correspond to the gas production caused by fermentation of the water-soluble components and those of sub-curve 2 (A2) to the gas production caused by fermentation of the non-soluble components (Cone *et al.*, 1997). The half-time value B2 is the incubation time (h) needed to reach half of A2.

Starch degradation after 10 h of incubation was calculated from the starch content and the amount of gas produced after 10 h, as described by Chai *et al.* (2004).

Experimental design and statistical analysis

Single plots of the different forage maize types were harvested and mechanically cut into 6-mm pieces of which 20 kg material was taken at random for chemical analysis and gas production incubation and ensiled in the 18-litre laboratory containers. From the not ensiled material as well as from the ensiled material duplicate samples were taken for analysis. Sources of variance (main effects and interactions) were analysed with an ANOVA (Analysis of Variance) procedure, resulting in F-probabilities of the sources of variance. 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

Chemical composition and digestibility

The results on DM content, ear proportion of the plants, chemical composition

Table 1. Chemical composition of the maize types Dry Down early ripening (DD-early), Stay Green early ripening (SG-early), Dry Down late ripening (DD-late) and Stay Green late ripening (DG-late), harvested on 20 August 2003 at a dry matter content of about 250 g kg^{-1}.

Maize type	DM	Ear[1]	Ash	CP*[2]	Starch	Sugar*	NDF*	ADF*	ADL*	OMD*	NDFD
	(g kg^{-1})	- - - - - - -	- - - - - - -	- - - - - - -	(g per kg DM)	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - -	(g per kg OM)	(g per kg NDF)
Entire plant											
DD-early	249	455	47	82	152	118	491	265	23	733	548
SG-early	254	512	54	79	183	117	468	247	21	755	566
DD-late	235	412	53	77	140	152	470	244	20	759	578
SG-late	247	446	55	83	193	105	450	234	20	752	531
Ear											
DD-early	350	·	19	·	402	·	·	·	·	·	·
SG-early	372	·	18	·	447	·	·	·	·	·	·
DD-late	327	·	18	·	375	·	·	·	·	·	·
SG-late	409	·	16	·	480	·	·	·	·	·	·
Stover											
DD-early	214	·	72	80	·	112	624	350	34	657	532
SG-early	227	·	81	77	·	133	695	334	31	677	524
DD-late	217	·	72	72	·	159	572	319	29	698	541
SG-late	215	·	86	82	·	111	622	355	34	657	508

[1] Ear is the fraction (g per kg DM) of the whole plant.

[2] * = contents determined with Near Infrared Reflectance Spectroscopy.

Table 2. Chemical composition of the maize types Dry Down early ripening (DD-early), Stay Green early ripening (SG-early), Dry Down late ripening (DD-late) and Stay Green late ripening (DG-late), harvested on 16 September 2003 at a dry matter content of about 320 g kg^{-1}.

Maize type	DM	Ear[1]	Ash	CP*[2]	Starch	Sugar*	NDF*	ADF*	ADL*	OMD*	NDFD
	(g kg^{-1})	- - - - - -	- - - - - -	- - - - - -	(g per kg DM)		- - - - - -	- - - - - -	- - - - - -	(g per kg OM)	(g per kg NDF)
Entire plant											
DD-early	348	586	42	72	364	42	391	211	20	757	534
SG-early	317	572	50	76	331	52	384	205	17	760	545
DD-late	320	552	46	71	321	76	397	211	17	767	564
SG-late	316	561	47	79	346	49	374	190	17	767	519
Ear											
DD-early	541	·	11	·	665	·	·	·	·	·	·
SG-early	527	·	14	·	593	·	·	·	·	·	·
DD-late	505	·	14	·	625	·	·	·	·	·	·
SG-late	559		13	·	636	·	·	·	·	·	·
Stover											
DD-early	242	·	76	65	·	64	694	410	44	593	498
SG-early	208	·	88	65	·	67	674	393	39	629	511
DD-late	227	·	81	53	·	123	663	388	39	640	524
SG-late	205	·	87	71	·	74	660	383	39	623	494

[1] Ear is the fraction (g per kg DM) of the whole plant.

[2] * = contents determined with Near Infrared Reflectance Spectroscopy.

and digestibility of OM and NDF of the maize plants harvested at an estimated DM content of 250, 320 or 390 g kg^{-1} on 20 August, 16 September and 3 October 2003, respectively, are summarized in Tables 1–3. The data show that at a DM content of 250 or 320 g kg^{-1} (Tables 1 and 2) the ear proportion was higher for the early-ripening types than for the late-maturing ones, but that the differences were smaller for the samples harvested at a DM content of 390 g kg^{-1} (Table 3).

Maturation caused dry matter content and whole-plant starch content to increase and the crude protein (CP), sugar, NDF and ADF contents as well as NDF digestibility (NDFD) to decrease. OM digestibility (OMD) did not change. The largest differences in chemical composition and digestibility were found between the samples harvested at a DM content of 250 and 320 g kg^{-1}. Further maturation from 320 to 390 g DM kg^{-1} reduced the differences.

In vitro rumen fermentation characteristics

Whole plants

The results on starch content and gas production parameters of whole plant samples (stover + ear) harvested at an estimated DM content of 250, 320 or 390 g kg^{-1} on 20 August, 16 September and 3 October, respectively, are summarized in Table 4. The

Table 3. Chemical composition of the maize types Dry Down early ripening (DD-early), Stay Green early ripening (SG-early), Dry Down late ripening (DD-late) and Stay Green late ripening (DG-late), harvested on 3 October 2003 at a dry matter content of about 390 g kg^{-1}.

Maize type	DM	Ear[1]	Ash	CP*[2]	Starch	Sugar*	NDF*	ADF*	ADL*	OMD*	NDFD
	(g kg^{-1}) - - - - - - - - - - - - - - -				(g per kg DM)	- - - - - - - - - - - - - - - - -				(g per kg OM)	(g per kg NDF)
Entire plant											
DD-early	401	595	41	64	353	36	430	238	22	745	492
SG-early	384	593	46	72	405	34	357	189	17	766	506
DD-late	389	582	43	67	379	47	368	193	17	769	520
SG-late	370	576	48	71	380	35	378	201	19	757	497
Ear											
DD-early	602	·	12	·	660	·	·	·	·	·	·
SG-early	586	·	12	·	649	·	·	·	·	·	·
DD-late	575	·	13	·	638	·	·	·	·	·	·
SG-late	613	·	13	·	643	·	·	·	·	·	·
Stover											
DD-early	268	·	75	38	·	100	701	410	43	610	477
SG-early	262	·	88	44	·	103	665	384	39	641	506
DD-late	249	·	88	41	·	131	645	381	39	642	503
SG-late	242	·	87	59	·	120	633	365	37	651	479

[1] Ear is the fraction (g per kg DM) of the whole plant.
[2] * = contents determined with Near Infrared Reflectance Spectroscopy.

data show that the DM content at harvest had a statistically significant ($P < 0.001$) influence on starch content: as expected, starch content increased with age. The DM content at harvest also had a statistically significant ($P < 0.01$ or lower) influence on all gas production parameters. Total gas production after 20 h of incubation (GP20) and gas production caused by fermentation of the non-soluble fraction (A2) increased with ripening, whereas the value for A1 decreased, indicating a lower gas production caused by fermentation of the soluble fraction. The maize types differed significantly ($P < 0.05$) in starch content, with highest contents for the Stay Green (SG) types, and in GP20 and A1, with highest values for late-ripening Dry Down maize (DD-late). Except for A1 ($P < 0.05$) there was no statistically significant DM content × maize type interaction.

Ears

The data on starch content and gas production characteristics of the ear samples are summarized in Table 5. DM content at harvest had a statistically significant ($P < 0.01$) influence on starch content and the investigated gas production parameters. The values for GP20, A2 and B2 increased and for A1 decreased with ripening. The calculated fraction of degraded starch after 10 h of incubation decreased with ripening. The effect of maize type on A1, A2 and calculated degraded starch was highly significant

Table 4. Gas production parameters [1] of not ensiled samples of entire maize plants of 4 maize types harvested at dry matter (DM) contents of about 250, 320 and 390 g kg^{-1}, on 20 August, 16 September and 3 October 2003, respectively.

DM content/ maize type	Starch	GP20	A1	A2	B2
	(g per kg DM)	--------	(ml per g OM)	----------	(h)
250 g DM kg^{-1}					
DD-early	152	242.8	52.0	190.8	7.65
SG-early	183	238.2	46.0	192.6	7.61
DD-late	140	256.0	65.7	190.3	7.70
SG-late	193	241.8	49.4	192.4	7.70
320 g DM kg^{-1}					
DD-early	364	256.4	30.6	225.8	7.56
SG-early	331	257.2	30.7	226.5	7.62
DD-late	321	270.6	41.2	229.4	7.62
SG-late	346	257.0	32.7	224.3	7.76
390 g DM kg^{-1}					
DD-early	353	247.8	25.1	222.7	7.79
SG-early	405	256.6	27.6	229.0	7.90
DD-late	379	263.8	31.1	232.7	7.66
SG-late	380	259.3	31.3	228.0	7.89
LSD[2]	·	10.4	5.5	6.5	0.21
250 g DM kg^{-1}	167	244.7	53.3	191.4	7.64
320 g DM kg^{-1}	341	260.3	33.8	226.5	7.64
390 g DM kg^{-1}	379	256.8	28.8	228.1	7.81
LSD	18	5.2	2.7	3.3	0.11
DD-early	290	249.0	35.9	213.1	7.67
SG-early	306	250.6	34.8	215.9	7.71
DD-late	280	263.4	46.0	217.5	7.63
SG-late	306	252.7	37.8	214.9	7.78
LSD	21	6.0	3.2	3.8	0.12
Statistical effects[3]					
DM content	***	***	***	***	**
Maize type	*	**	***	ns	#
DM × type	·	ns	*	ns	ns

[1] GP20 = gas production after 20 h of incubation; 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 needed to reach half of A2.

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

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

Table 5. Gas production parameters[1] of ear samples of four maize types. Ears were sampled at dry matter (DM) contents of the whole maize plant of about 250, 320 and 390 g kg^{-1}, on 20 August, 16 September and 3 October 2003, respectively. The degraded starch fraction after 10 h of incubation was calculated according to Chai *et al.* (2004).

DM content/ maize type	Starch	GP20	A1	A2	B2	Degraded starch
	(g per kg DM) - - - - - - -		(ml per g OM)	- - - - - -	(h)	(g per kg)
250 g DM kg^{-1}						
DD-early	402	290.2	47.1	243.1	7.09	776
SG-early	447	311.9	44.9	267.0	7.31	785
DD-late	375	301.0	54.1	246.9	7.28	818
SG-late	480	291.8	36.4	255.4	7.32	704
320 g DM kg^{-1}						
DD-early	665	306.9	27.9	279.0	7.31	639
SG-early	593	315.2	29.4	285.8	7.68	679
DD-late	625	309.1	31.6	277.5	7.52	659
SG-late	636	310.4	24.0	286.4	7.67	644
390 g DM kg^{-1}						
DD-early	660	313.9	23.9	290.0	7.80	632
SG-early	649	309.8	22.1	287.8	7.83	617
DD-late	638	304.8	25.3	279.6	7.54	627
SG-late	643	307.4	24.6	282.8	7.93	602
LSD[2]	.	10.4	3.8	8.3	0.46	26
250 g DM kg^{-1}	426	298.7	45.6	253.1	7.25	771
320 g DM kg^{-1}	630	310.4	28.2	282.2	7.54	665
390 g DM kg^{-1}	648	309.0	23.9	285.0	7.77	619
LSD	29	5.2	1.9	4.2	0.23	13
DD-early	576	303.6	32.9	270.7	7.40	682
SG-early	563	312.3	32.1	280.2	7.61	694
DD-late	546	305.0	37.0	268.0	7.44	701
SG-late	586	303.2	28.3	274.9	7.64	650
LSD	33	6.0	2.2	4.8	0.27	15
Statistical effects[3]						
DM content	***	***	***	***	**	***
Maize type	#	*	***	***	ns	***
DM × type	.	*	**	*	ns	**

[1] GP20 = gas production after 20 h of incubation; 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 needed to reach half of A2.

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

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

Table 6. Gas production parameters[1] of stover samples of 4 maize types. The stover was sampled at dry matter (DM) contents of the whole maize plant of about 250, 320 and 390 g kg^{-1}, on 20 August, 16 September and 3 October 2003, respectively.

DM content/ maize type		GP20	A1	A2	B2
		--------	(ml per g OM)	----------	(h)
250 g DM kg^{-1}					
DD-early		204.4	47.5	156.9	8.70
SG-early		203.4	46.3	157.1	8.58
DD-late		225.8	64.3	161.6	8.23
SG-late		206.3	50.3	156.0	8.37
320 g DM kg^{-1}					
DD-early		184.0	34.1	149.9	9.63
SG-early		187.8	32.2	155.6	8.90
DD-late		200.4	45.8	154.6	8.92
SG-late		199.9	41.0	159.0	8.76
390 g DM kg^{-1}					
DD-early		182.3	38.8	143.5	9.68
SG-early		188.6	39.2	149.5	9.39
DD-late		207.0	48.4	158.6	9.38
SG-late		184.9	46.9	138.0	9.17
	LSD[2]	12.5	5.7	9.6	0.30
250 g DM kg^{-1}		210.0	52.1	157.9	8.47
320 g DM kg^{-1}		193.0	38.3	154.8	9.05
390 g DM kg^{-1}		190.7	43.3	147.4	9.40
	LSD	6.3	2.9	4.8	0.15
DD-early		190.2	40.1	150.1	9.34
SG-early		193.2	39.2	154.0	8.96
DD-late		211.1	52.8	158.3	8.84
SG-late		197.0	46.1	151.0	8.76
	LSD	7.2	3.3	5.6	0.17
Statistical effects[3]					
DM content		***	***	**	***
Maize type		***	***	*	***
DM × type		ns	ns	#	#

[1] GP20 = gas production after 20 h of incubation; 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 needed to reach half of A2.

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

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

Table 7. Gas production parameters[1] of ensiled maize samples of 4 maize types. Samples of whole plants harvested at dry matter (DM) contents of about 250, 320 and 390 g kg^{-1}, on 20 August, 16 September and 3 October 2003, respectively. The degraded starch fraction after 10 h of incubation was calculated according to Chai *et al.* (2004).

DM content/ maize type		Starch (g per kg DM)	GP20 - - - - -	A1 (ml per g OM)	A2 - - - - - -	B2 (h)	Degraded starch (g per kg)
250 g DM kg^{-1}							
DD-early		178	220.9	36.5	184.4	8.72	616
SG-early		192	231.3	34.9	196.4	8.47	659
DD-late		147	235.3	37.4	197.9	8.53	796
SG-late		199	229.2	29.2	199.3	8.35	679
320 g DM kg^{-1}							
DD-early		339	252.8	23.8	229.0	8.37	616
SG-early		343	241.1	21.4	219.7	8.23	569
DD-late		314	251.4	26.2	225.3	8.18	643
SG-late		329	241.4	26.5	214.7	8.00	628
390 g DM kg^{-1}							
DD-early		364	246.4	20.0	226.4	8.38	570
SG-early		343	247.3	18.6	228.7	8.20	597
DD-late		347	258.1	23.7	234.5	8.02	651
SG-late		354	234.5	21.4	213.2	7.96	568
	LSD[2]	·	11.8	5.7	11.1	0.65	97
250 g DM kg^{-1}		179	229.1	34.7	194.5	8.51	687
320 g DM kg^{-1}		331	246.6	24.4	222.2	8.19	614
390 g DM kg^{-1}		352	246.6	20.9	225.7	8.14	596
	LSD	11	5.9	2.8	5.5	0.32	49
DD-early		294	240.0	26.8	213.3	8.49	600
SG-early		293	239.9	25.0	214.9	8.30	608
DD-late		269	248.3	29.1	219.1	8.24	697
SG-late		294	234.9	25.9	209.0	8.10	625
	LSD	13	6.8	3.3	6.4	0.37	56
Statistical effects[3]							
DM content		***	***	***	***	#	**
Maize type		**	**	#	*	ns	*
DM × type		·	#	ns	*	ns	ns

[1] GP20 = gas production after 20 h of incubation; 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 needed to reach half of A2.

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

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

($P < 0.001$), although in some cases the differences were rather small. A statistically significant ($P < 0.05$) DM content × maize type interaction was only found for A1 and for the calculated fraction of degraded starch.

Stover

The data for the gas production characteristics of the stover samples are presented in Table 6. DM content at harvest (ripening stage) had a statistically significant ($P < 0.01$ or lower) influence on the gas production parameters investigated. GP20, A1 and A2 decreased and B2 increased with ripening, which indicated a lower rate of fermentation. Maize type had a statistically significant ($P < 0.05$ or lower) effect on all gas production parameters. Except for B2 the values were highest for DD-late. No statistically significant ($P < 0.05$) DM content × maize type interaction was found.

Ensiled plants

The data on starch content and gas production parameters for the ensiled maize plants (stover + ear) harvested at the three stages of maturity are listed in Table 7. DM content at harvest had a statistically significant ($P < 0.001$) effect on starch content and the gas production parameters investigated, except for B2. GP20 and A2 increased and A1 and B2 decreased with ripening. The calculated proportion of degraded starch after 10 h of incubation in rumen fluid decreased with ripening. Maize type had a statistically significant effect on starch content and GP20 ($P < 0.01$) and on A2 and the proportion of degraded starch ($P < 0.05$). There was a statistically significant ($P < 0.05$) DM content × maize type interaction only for A2.

Discussion

Chemical composition

The three harvest dates (ripening stages) were chosen such that the plants would have dry matter contents of on average 250, 320 or 390 g kg^{-1}. However, the data in Tables 1–3 show that the desired values were not reached exactly. The DM contents of 250 and 390 g kg^{-1} are at the extreme ends of what is usual in the Netherlands. The DM content of the total plant does not *per se* reflect the ear proportion as the DM content of the whole plant is determined by the proportions in the fresh weight of ear and stover and their respective DM contents (Struik, 1983; Eder, 1999). It was expected that the DM content of the stover of the Stay Green types would be lower than that of the Dry Down types (Schlagheck *et al.*, 2000; Schwarz & Ettle, 2000), but this was not the case (Tables 1–3). Only at a DM content of the total plant of 320 g kg^{-1} (harvested on 16 September) the DM content of the stover of the Stay Green types (Table 2) was little lower than that of the Dry Down types, but this difference had virtually disappeared at a total plant DM content of 390 g kg^{-1} (3 October) (Table 3). No clear differences in DM content of the stover were observed between the early- and late-ripening types either (Tables 1 and 2). Only on 3 October (Table 3), when the maize plants were ripe, a small difference in DM weight of the stover was recorded in favour of the early-ripening types. The development of the maize plants depends largely on environmental conditions, such as temperature, day length, sunshine, water availability (Struik, 1983; Meisser & Wyss, 1998) and plant density (Bavec & Bavec, 2002). The year in which the plants were grown was characterized by a wet spring followed by an extremely warm and dry summer. It could be that these, for the Netherlands extreme conditions nullified the expected differences, although all plants were harvested at comparable DM contents. Muchow (1990) showed that increasing the daily temperature from 25 to 32 °C increased the rate of grain growth and decreased the effective period of grain filling. The largest differences in chemical composition were found between plants harvested at DM contents of 250 and 320 g kg^{-1} (20 August and 16 September). During this period the average starch content of the whole plant increased from 167 to 341 g per kg DM. The contents of all other chemical components decreased because of dilution with the increasing amount of starch (Russell, 1986; Argillier & Barrière, 1996; Deaville & Givens, 2001). During ripening from 320 to 390 g DM kg^{-1} (16 September – 3 October) changes in chemical composition were relatively small. During this period the starch content of the total plant increased from 341 to only 379 g per kg DM. Philippeau & Michalet-Doreau (1997) observed no changes in starch content during prolonged maturation at a DM content above 350 g kg^{-1}. Also Struik (1983) and Cone & Engels (1993) showed that after mid August the chemical composition of the cell walls in the stem does not change much.

Degradability of organic matter and neutral detergent fibre

Average organic matter digestibility (OMD) of the whole plants with DM contents of 250 to 390 g kg^{-1} (20 August – 3 October) varied between 750 and 763 g per kg OM. Despite major changes in the carbohydrate composition of the plants, whole plant apparent digestibility may change little during maturation (Browne *et al.*, 1999) or may even decrease slightly. Russell (1986) observed a slight decrease in the content of *in vitro* digestible dry matter during a maturation period of 60 days. Average OMD of the stover ranged from 672 (250 g DM kg^{-1}) to 621 (320 g DM kg^{-1}) and to 636 g per kg OM (390 g DM kg^{-1}). Also here major changes were seen when DM content increased from 250 to 320 g kg^{-1} (20 August – 16 September). During ripening, NDF degradability (NDFD) of the whole plant decreased gradually from 556 to 504 g per kg NDF and of the stover from 526 to 491 g per kg NDF. These results are in agreement with results of Deaville & Givens (2001) investigating maize silages harvested at DM contents of 228, 273, 304 or 372 g per kg DM, and with results of Flachowsky *et al.* (1993). Findings of Cone & Engels (1993) show a gradual decrease in stem cell wall degradability from July to the end of August, after which degradability remained more or less constant. The results in Tables 1–3 show no systematic influence of maize type on OM and NDF degradability. Givens & Deaville (2001) concluded that new forage maize varieties, being earlier maturing, have higher DM and starch contents at the same harvest date, at the expense of lower cell wall digestibility with little overall change in OM digestibility. In the present study no systematic influence of early or late ripening on OM and NDF degradability was observed. Based on their research, Givens & Deaville (2001) also concluded that cell wall digestibility is more related to cell wall content, raising the possibility that NDF content is a better index of physiological maturity than DM content.

In vitro rumen fermentation characteristics

DM content at harvest had a statistically significant influence on the fermentation characteristics of the entire plants, the ears and the stover (Tables 4–6). In several instances major changes were observed during the period that DM content increased from 250 to 320 g kg^{-1} (20 August – 16 September) and only minor changes during prolonged ripening. This is in agreement with the findings of Cone & Engels (1993) for cell wall degradability. Our results (Tables 4–6) show that the level of fermentation (GP20, A1, A2) and the rate of fermentation (B2) were influenced by the ripening stage. GP20 and A2 for both the entire plant and the ears increased with ripening (Tables 4 and 5) as a result of the increased starch content. At the same time the values of A1 decreased as a result of the lower sugar contents (Tables 1–3). The rate of fermentation (B2) gradually decreased (higher values of B2) with ripening (Tables 4 and 5) because of the decreased rate of degradability of the cell walls in the stover (Table 6) (Struik, 1983; Cone & Engels, 1993). Using the method of Chai *et al.* (2004) to calculate starch degradation it was shown that starch degradation decreased from 771 g per kg at a total plant DM content of 250 g kg^{-1} (20 August) to 619 g per kg starch (390 g DM kg^{-1}; 3 October). Comparable results were obtained by Loose (2000), whereas others found less clear effects of the stage of maturation on starch degradability (Ettle & Schwarz, 2003). Upon prolonging maturation from 22 to 78 days post-silking, Philippeau & Michalet-Doreau (1997) found a decreased content of rapidly degradable starch and an increased content of slowly degradable starch. At the same time the rate of degradation as determined with the *in situ* nylon bag technique markedly decreased, which resulted in a large influence of harvest date on the effective degradability of starch. These authors observed a less pronounced influence of the maize types dent and flint.

Maize types

Although in several instances a statistically significant effect was found of maize type on the fermentation characteristics for the entire plants, the ears and the stover, the effect of none of the maize types (Stay Green, Dry down, early, late) was systematic. Also Schlagheck *et al.* (2000) found no influence of the maize types Stay Green and Dry Down, harvested at comparable DM contents, on the *in vitro* degradation of entire plants and ears, but they did find differences for the ear-free stover. Russell *et al.* (1992) found only small differences in *in vitro* digestible dry matter between early, medium and late-maturing hybrids, but instead of harvesting the plants at exactly the same DM contents, they harvested at a fixed period after silking. Also Irlbeck *et al.* (1993) observed a decreasing content of *in vitro* digestible dry matter caused by prolonged maturation from 50 to 78 days post-silking and a slightly lower content of *in vitro* digestible dry matter for the later maturing hybrids compared with the earlier maturing ones.

Ensiled maize

Comparing the fermentation characteristics of the entire maize plants after ensiling (Table 7) with the results in Table 4 for not ensiled entire plants show that gas production of the ensiled plants was slightly lower than that of the not ensiled plants, mainly because of lower A1 values. Obviously, the soluble sugars had already been fermented during the ensiling process. Despite these differences the results for the ensiled plants were comparable with the not ensiled plants. Irlbeck *et al.* (1993) found similar results. Also the results in Table 7 show main effects of DM content at harvest and in some cases statistically significant influences of maize type. Also for the ensiled plants no systematic influences of one of the maize types was found. Only the estimated starch degradation, based on starch content and gas production (Chai *et al.*, 2004), was systematically higher for the late-ripening than for the early-ripening types.

Conclusions

- DM content at harvest, and so harvest date of the maize plants had large effects on chemical composition, digestibility and *in vitro* determined fermentation characteristics. The effects became more pronounced in the early stages of ripening when DM increased from 290 to 320 g kg^{-1} (20 August – 16 September) than during prolonged ripening (up to 390 g DM kg^{-1}; 3 October).
- Maize type (Dry Down, Stay Green, early ripening, late ripening) had no systematic effect on chemical composition, digestibility and *in vitro* rumen fermentation characteristics. Claimed differences between Dry Down and Stay Green types and between early and late-ripening types were not confirmed.

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