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Research Article

Comparison of *In Vitro* Dry Matter Degradation of Four Maize Silages Using the Menke Gas Production Method

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Abstract: Four maize silages from the UK and France were evaluated using the gas production method to estimate the rate and extent of dry matter degradation *in vitro*. There were significant differences in gas production after 17 h incubation. The silages showed similar times to produce 50, 95 and 100% of total gas production, whereas the lag time for Ema was significantly ($P < 0.001$) lower than that for Volgate. The potential gas production value (a + b) for Nancis was significantly ($P < 0.05$) lower than that for Volgate.

There was a significant correlation ($r = 0.996$, $R^2 = 90.71$, $P < 0.001$) between gas production and total VFA production. There were considerable differences in the fermentability of carbohydrates between different maize silages. Nancis and Ema had more readily fermentable carbohydrate, which yielded high propionate production and a shorter lag time. 6196 and Volgate had more slowly fermentable carbohydrate, which did become available to microorganisms but after a longer lag time.

Key Words: Maize silage, Gas production, VFA production

Menke Gaz Üretim Tekniği ile Dört Farklı Mısır Silajının in vitro Kuru Madde Parçalanmasının Karşılaştırılması

Özet: İngiltere ve Fransa'da yetiştirilen mısırdan yapılan 4 silajın in vitro gaz üretim methodu kullanarak kuru maddenin parçalanma hızı ve miktarı tahmin edilmiştir.

17 saatlik inkubasyondan sonra üretilen gaz miktarı mısır çeşitlerine göre önemli farklılıklar göstermiştir ($P < 0.001$). Toplam gazın% 50, 95 ve 100'ünü üretmek için geçen süre bütün silajlar için aynı olmasına rağmen, gaz üretiminin başlaması için geçen süre (lag time) silaj Ema için, silaj Volgateden önemli derecede kısa olmuştur ($P < 0.001$). Ayrıca silaj Nancis için potensiyel gaz üretimi, silaj Volgateden daha az bulunmuştur ($P < 0.05$).

Üretilen gaz ile üretilen uçucu yağ asitleri arasında önemli bir korelasyon bulunmuştur ($r = 0.996$, $P < 0.001$). Silaj karbonhidratlarının fermente olma şekilleri arasında önemli farklılıklar olup silaj Nancis ve Ema' da bulunan karbonhidratlar mikroorganizmalar tarafından daha kolay bir şekilde kullanılmıştır. Sonuç itibariyle yüksek miktarda propiyonik asit üretimine neden olmuştur.

Anahtar Sözcükler: Mısır silajı, Gaz üretimi, Uçucu yağ asitleri

Introduction

Fermentation of feedstuffs with buffered rumen fluid yields short chain volatile fatty acids (VFA), gasses (mainly CO_2 and CH_4) and microbial cells. The production of gas during fermentation has been used as an indirect measure of substrate degradability (1). The gas produced is formed directly as a result of fermentation (CO_2 and CH_4) and the gas produced indirectly from the buffering of VFA (CO_2 released through the bicarbonate buffer) (2).

The gas production is primarily the result of the fermentation of carbohydrate to VFA (3-5) since gas produced from protein fermentation is relatively small compared to that of carbohydrate fermentation (3) and the contribution of fat metabolism to gas production is negligible (2). At present, cumulative gas production is increasingly being used to measure the fermentation kinetics of ruminant feeds (4,6).

The aim of this experiment was to determine the dry matter degradability of the four maize silages using the Menke gas production technique.

Materials and Methods

Chemical analysis

Four maize hybrids from different sites within the UK [Nancis and Ema] and France [6196 and Volgata] were ensiled in sealed drums with no additives. The dry matter and nutrient contents of each silage are given in Table 1.

The pH of silages was determined using a combination electrode of a pH meter (Pye UNICAM, PHILLIPS). Water-soluble carbohydrate (WSC) contents were determined by the modified anthrone method (7). Oven dry matter contents were determined by drying accurately weighed samples of silage in aluminium foil trays at 105 ºC for 24 h using a fan-assisted oven. Nitrogen contents were determined using the Leco Fp428 Nitrogen Analyser. Neutral detergent fibre (NDF) contents were determined by the modified method of Van Soest et al. (8). Starch contents were determined by the modified method of MacRae and Armstrong (9).

In Vitro Gas Production

The silage samples were oven dried at 60 ºC for 48 h and ground to pass through a 1 mm sieve. Approximately 0.2 g of sample was incubated in a bottle containing 50 ml of McDougall's buffer/rumen fluid mixture (10). The bottles were stoppered with rubber and crimp sealed. The incubation was carried out in a water bath at 39 ºC. The bottles were shaken 30 min after the start of incubation and then at 3 h intervals for 12 h. Triplicates of each sample were used in two separate runs. Three bottles containing only rumen fluid/buffer solution were included with each run and the mean gas production value of these bottles was termed the blank value. The blank value was subtracted from each measurement to give the net gas production. Readings of gas production were recorded at 3, 7, 17, 24, 48 and 72 h. The measurements of VFA production were performed at 7, 17 and 24 h incubation, which was the period of maximum fermentation based on the data from preliminary experiments.

Cumulative gas production data were fitted to the model of Orskov and McDonald (11)

$y = a + b\,(1-e^{-ct})$ (11,12,13)

where

a = the gas production from the immediately soluble fraction

b = the gas production from the insoluble fraction

c = the gas production rate constant for the insoluble fraction (b)

t = incubation time

(a + b) = the potential extent of gas production

y = gas produced at time 't'

Cumulative gas production data were also fitted to the model of France et al. (14) using the Most Likelihood Program (15).

$y = A - BQ^{t}Z^{\sqrt{t}}$ (12)

where

$Q = e^{-b}$, $Z = e^{-c}$, and $B = e^{\,bT + c\sqrt{T}}$ where y denotes cumulative gas production (ml), t is incubation time (h), A is the asymptotic gas pool size (ml), T is the lag time and b (h^{-1}) and c ($h^{-0.5}$) are fractional rate constants. A combined fractional rate (h^{-1}) of gas production (m) was calculated as $\mu = b + c / 2\sqrt{t}$ where t is incubation time (h).

Table 1. Composition of Silage from Four Maize Hybrids.

Constituents	Silages			
	Nancis	Ema	6196	Volgate
DM (g/kg FWt)	270	250	420	580
Starch (g/kg DM)	245	250	348	350
WSC (g/kg DM)	79	80	50	37
Protein (g/kg DM)	86	96	85	86
NDF (g/kg DM)	410	383	353	384
pH	3.41	3.46	3.78	4.26

The output from this program consists of estimates of the lag time (L), the time to produce 50% of gas pool, the time to produce 95% of gas pool and the total potential gas pool size (ml).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model. Significant differences between individual means were identified using Tukey's multiple range test (16). Differences were considered to be significant if $P < 0.05$. Regression analysis was performed to assess the relationship between gas production and VFA production.

Results

Gas Production

Data of gas production during the fermentation period are shown in Table 2. The cumulative volume of gas production increased with increasing time of incubation. Gas produced at 72 h incubation ranged between 49.7 and 53.4 ml per 0.2 g of substrate. There were no significant ($P > 0.05$) differences between silages with regard to gas production after 17 h incubation. By 24 h of incubation the total volume of gas produced was different for the different silages. The volume of gas production for Nancis was significantly ($P < 0.001$) lower than those for 6196 and Volgate at 24, 48 and 72 h incubation; but there was no significant ($P > 0.05$) difference between Nancis and Ema or 6196 and Volgate.

Values for the estimated parameters obtained from the kinetic models of France et al. (12) and Orskov and McDonald (11) are given in Table 3. There was no significant ($P > 0.05$) difference between silages with regard to the time to produce 50% and 95% of gas pool or total gas pool size. The lag time for Ema was significantly ($P < 0.05$) lower than those for 6196 and Volgate. The lag time for Nancis was not significantly ($P < 0.05$) different from that of Ema, and there was also no significant ($P > 0.05$) difference in the lag time between 6196 and Volgate. The four silages had similar values for constants a and c although the average values for constant b for Volgate were significantly ($P < 0.001$) higher than that for Nancis. The average potential gas production (a + b) value for Nancis was significantly ($P < 0.05$) lower than that for Volgate.

Volatile Fatty Acids

Data of total VFA, acetic, propionic acid and the acetic to propionic ratio are given in Table 4. The total VFA, acetic and propionic acid concentration (mM) increased with increasing incubation time.

At 7 h incubation, the propionic acid and total VFA concentrations (mM) of the incubation mixture for Nancis and Ema were significantly ($P < 0.001$) higher than those for 6196 and Volgate. At 17 h, the average acetic and propionic acid concentrations for all silages were not significantly ($P > 0.05$) affected by the type of silage. At 24 h incubation the acetic acid, propionic acid and total VFA concentration for Volgate were significantly ($P < 0.001$) higher than those for Nancis and Ema. The proportion of acetic acid for Nancis and Ema increased

Table 2. The Effect of Incubation Time on Gas Production.

	SILAGES					
Time (h)	NANCIS	EMA	6196	VOLGATE	Sig.	SEM
3	4.66	5.00	4.83	4.83	NS	0.241
7	10.16	10.16	9.66	9.16	NS	0.385
17	23.17	24.00	23.83	23.67	NS	0.353
24	33.00^{a}	33.17^{ab}	34.83^{bc}	35.50^{c}	***	0.439
48	42.00^{a}	41.67^{a}	44.83^{b}	44.00^{b}	***	0.318
72	49.67^{a}	52.50^{ab}	53.00^{b}	53.33^{b}	*	0.753

Means within the same row with differing superscripts are significantly different. SEM: Standard error of the mean. NS: Non-significant *** $P < 0.001$, * $P < 0.05$

Table 3. The Effect of Incubation Time on Estimated Parameters.

Estimated Parameters	SILAGES					
Time (h)	NANCIS	EMA	6196	VOLGATE	Sig.	SEM
Lag time (h)	0.29[ab]	0.23a	0.52[ab]	0.64[b]	***	0.098
50% (h)	19.54	22.22	19.48	19.40	NS	0.794
95% (h)	79.55	90.66	76.37	75.36	NS	4.585
Gas Pool size (ml)	53.11	56.31	55.52	55.36	NS	1.066
a	-0.508	-0.335	0.955	-1.067	NS	0.159
b	54.90[a]	56.76[ab]	58.58[ab]	58.69[b]	***	0.780
c	0.034	0.032	0.034	0.034	NS	0.001
a + b	54.39[a]	56.42[ab]	57.63ab	57.63[b]	*	0.860

Means within the same row with differing superscripts are significantly different. SEM: Standard error of mean. NS: Non-significant *** P < 0.001, * P < 0.05

Table 4. The Effect of Dietary Treatment and Incubation Time on VFA Concentration.

	SILAGES					
Time (h)	NANCIS	EMA	6196	VOLGATE	Sig.	SEM
		Acetic Acid				
7	6.06ab	6.40[a]	5.55[ab]	5.41[b]	***	0.253
17	9.36	9.39	8.87	8.81	NS	0.263
24	11.81[a]	12.31[a]	13.38[ab]	14.25[b]	***	0.463
		Propionic Acid				
7	4.17[a]	4.38[a]	3.33[b]	3.41[b]	***	0.120
17	5.87	5.88	6.53	6.152	NS	0.187
24	6.87[a]	7.14[a]	8.40[b]	8.69[b]	***	0.226
		Total VFA				
7	11.89[a]	12.48[a]	10.02[b]	9.68[b]	***	0.423
17	17.90	17.92	17.20	16.59	NS	0.503
24	21.65[a]	23.01[a]	24.82[ab]	25.83[b]	***	0.825
		Acetic acid:Propionic acid ratio				
7	1.452[a]	1.453[a]	1.681[b]	1.586[c]	***	0.029
17	1.595[a]	1.596[a]	1.360[b]	1.430[c]	***	0.012
24	1.720[a]	1.719[a]	1.590[b]	1.637[ab]	***	0.022
		Proportion of Acetic Acid				
7	0.509[a]	0.511[a]	0.558[b]	0.559[b]	***	0.004
17	0.522[a]	0.522[a]	0.516[ab]	0.531[c]	NS	0.002
24	0.545[a]	0.535[b]	0.538[ab]	0.551[c]	NS	0.002
		Proportion of Propionic Acid				
7	0.351[a]	0.353v	0.339[b]	0.353[c]	NS	0.004
17	0.328[a]	0.328[a]	0.379[b]	0.370[b]	***	0.004
24	0.317[a]	0.312[a]	0.339[b]	0.337[b]	***	0.003

Means within the same row with differing superscripts are significantly different. SEM: Standard error of the mean NS: Non-significant. *** P < 0.001

with increasing time of incubation, while the propionate acid proportion decreased. At 7 h incubation, the acetic acid proportion for Nancis and Ema was significantly ($P < 0.001$) lower than those for 6196 and Volgate, whereas the propionic acid proportion for Nancis and Ema was significantly ($P < 0.001$) higher than those for 6196 and Volgate. The acetic to propionic acid ratio for Nancis and Ema was significantly ($P < 0.001$) lower than those for 6196 and Volgate at 7 h incubation.

The proportion of acetic acid for 6196 and Volgate decreased between 7 and 17 h of incubation with a corresponding increase in propionate acid, but by 24 h incubation the proportions of acetic and propionic acids had returned to the initial levels. The acetic to propionic ratio for Nancis and Ema increased with increasing time of incubation. On the other hand, the ratio for 6196 and Volgate decreased between 7 and 17 h of incubation, but after 24 h incubation the ratio returned to the initial level.

Discussion

Gas production is associated with VFA production following fermentation of substrate so the more fermentable the substrate the greater the gas production, although the fermentation end products do influence the value of gas produced. Therefore total VFA production should correlate more closely with gas production than dry matter loss (5).

6196 and Volgate gave more gas than Nancis and Ema. Differences between total gas production could be explained by the differences in total VFA production and molar proportions of VFA (4).

The mean total gas production after 72 h incubation for the four maize silages was approximately 5 ml lower than that reported by Valentin et al. (17) for maize silages. The fermentation of protein yields ammonia, which combines with H + from the buffer to form NH_4 + , which remains in solution thus inhibiting indirect gas production. The concentration of ammonium ion in solution was high due to provision of peptone in the medium as a readily available N source for microbes. The ammonia would have prevented the release of CO_2.

The changes in VFA production may be due to the differences in the amount and nature of carbohydrates available to microorganisms. The amount of starch in Nancis and Ema was considerably less than those in 6196 and Volgate. After 10 h incubation the starch content of Nancis and Ema might have been fully utilised while some of the starch in 6196 and Volgate may have been still available to rumen microorganisms to produce VFA. Eventually more acetic and propionic acids and total VFA were produced from 6196 and Volgate.

Another reason for high propionate production (mM) for Nancis and Ema at short fermentation times might be the difference in the fermentability of available carbohydrates. The water-soluble carbohydrate contents of Nancis and Ema were considerably higher than those for 6196 and Volgate. Getachew et al. (2) reported that rapidly fermentable carbohydrates produce relatively higher propionate as compared to acetate and the reverse takes place when slowly fermentable carbohydrates are fermented.

As can be seen from Figure 1 there was a significant correlation ($r = 0.996$, $R2 = 90.71\%$ $P < 0.001$) between gas production and total VFA production. This is in agreement with the findings of Doane et al. (18), who found a significant correlation between gas and VFA production.

The lag time (time from incubation to start of gas production), time to produce 50% of the maximum gas production and time to produce 95% of the maximum gas production are very important digestibility parameters. A substrate which is degraded more effectively results in a shorter time to produce 50% and 95% of maximum gas production. It will remain in the rumen for a shorter period and supply more nutrients to the animals. The shorter lag time period for Nancis and Ema could be due to high WSC content. There was no data relating to lag time, or time to produce 50% and 95% of gas production of the gas pool for maize silage in the literature with which to compare the results of this

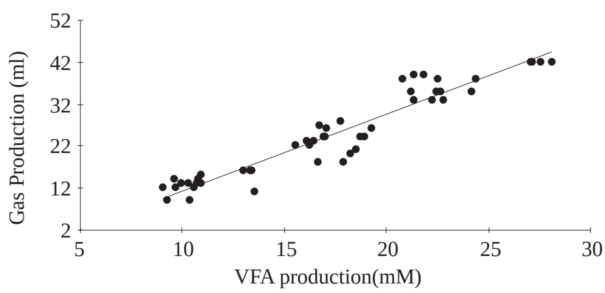

Figure 1. The Relationship between Cumulative Gas Production and VFA Production.

experiment. Therefore, the gas production data obtained by Valentin et al. (17), who used 10 different maize silages, has been processed in the same way as our data The lag time, and time to produce 50% and 95% of gas production of gas pool in the current experiment were considerably higher than those estimated from the data of Valentin et al. (17). This may be due to a difference in the dilution of rumen fluid. The rumen fluid in the current experiment was diluted four times while the dilution factor was two in the experiment carried out by Valentin et al. (17). The c value obtained after processing the data obtained by Valentin et al (17) was two - fold higher than the value obtained in the current experiment. Therefore, the shorter lag time and higher c values resulted in a shorter time to produce 50% and 95% of gas pool in their experiment. The b, (a + b) and gas pool size values calculated from the data of Valentin et al. (17) were 5 ml higher than ours. The a value calculated from data obtained by Valentin et al. (17) was similar to those for Nancis and Ema but lower than that for Volgate.

In conclusion, there are considerable differences in the fermentability of carbohydrates between different maize silages. Nancis and Ema with low dry matter had a more readily fermentable carbohydrate, which yielded high propionate production and a shorter lag time (time to start gas production). 6196 and Volgate had more slowly fermentable carbohydrate, which did become available to microorganisms but after a longer lag time.

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