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Research Article

Determination of Microbial Gas Production, Fermentation Kinetics and Digestibility of Alternative Crop Silages

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Abstract: Microbial gas production (MGP), fermentation kinetics and DM loss of crop silages made from 7 different plant families (barley/pea, clover, grass, kale, lotus, lucerne, sainfoin) and 10 different refusals were determined. The pressure transducer technique (PTT) was used to measure the microbial gas production of fresh and ground silages and refusal samples at regular intervals throughout the 120 h incubation. The MGP of fresh and ground silages were similar (r^2 = 0.90). The maximum gas production was obtained from kale for ground and fresh silages whilst grass silage yielded the maximum gas pool for refusals. No differences were found between lag time but times to produce 50% and 95% gas pool were shorter with ground silages than with fresh silages and refusals. Total volatile fatty acid (VFA) production was notably lower for refusals compared to fresh and ground silages, yet ground and fresh silages yielded similar VFA levels. Dry matter (DM) losses of ground and fresh silages were similar. However, lower DM losses were observed for refusals. These results suggest that refusals could be used as a valuable and digestible feed-stuff for animals.

Key Words: Microbial gas production, fermentation kinetics, silage digestibility

Alternatif Ürün Silajlarının Sindirilebilirliğinin, Fermentasyon Kinetiklerinin ve Mikrobiyal Gaz Üretiminin Belirlenmesi

Özet: Yedi farklı bitki familyasından yapılmış silajların (arpa, üçgül, çim, kolza, gazal boynuzu, kaba yonca, korunga) ve bunlara ait 10 farklı yemin, tercih edilmeyen kısmının (refusal) kuru madde kaybına dayalı sindirilebilirliği, fermentaston kinetiği ve mikrobiyal gaz üretimi belirlenmiştir. Mikrobiyal gaz üretimi taze ve öğütülmüş silajlar ve artık yem örnekleri kullanılarak 120 saatlik inkübasyonun belirli dönemlerinde basınç ölçme tekniği (PTT) ile tayin edilmiştir. Taze ve öğütülmüş silajların mikrobiyal gaz üretimi birbirine benzer bulunmuştur (r^2 = 0,90). Öğütülmüş ve taze silajlarda kolza en fazla mikrobiyal gaz üretimine neden olurken tercih edilmeyen yemler için en fazla mikrobiyal gaz üretimi çim silajında gerçekleşmiştir. Farklı silajlara ait lag süreleri arasında bir farklılık gözlenmemiş fakat gaz üretiminin % 50 ve % 95'e ulaşma zamanı öğütülmüş silajlarda taze ve tercih edilmeyen kısm silajlara oranla daha kısa bulunmuştur. Toplam uçucu yağ asitlerinin üretimi artık yemlerde taze ve öğütülmüş silajlara oranla daha az gerçekleşmiş fakat taze ve öğütülmüş silajlar arasında uçucu yağ asitleri üretimi bakımından farklılık gözlenmemiştir. Kuru madde (KM) kaybı öğütülmüş ve taze silajlarda benzerlik göstermesine ragmen artık yemlerdeki KM kaybı daha az düzeyde gerçekleşmiştir. Bu sonuçlar tercih edilmeyen yemin hayvanlar tarafından kullanılabilecek değerli ve sindirilebilir bir yem kaynağı olduğunu göstermiştir.

Anahtar Sözcükler: Mikrobiyal gaz üretimi, fermentasyon kinetiği, silaj sindirilebilirliği

Introduction

During the last 3 decades, measurement of in vitro microbial gas production (MGP) has received great impetus and become increasingly popular for determining plant digestion characteristics and the kinetics of fermentation (1-3). Although plants are an abundant and renewable source of organic energy, less than 50% of the biomass may be degraded by mammalian digestive enzymes. This indigestible plant biomass, which mainly consists of structural polysaccharides, can be biodegraded in the rumen by protozoa (4), bacteria (5) and fungi (6) in a unique niche. Gases (CO_2 and CH_4) and volatile fatty acids (VFAs) including acetate, butyrate and propionate are produced as end products of microbial fermentation of ingested plant materials in the rumen. Valeric acid is also produced in batch culture (7,8). Gas production from protein fermentation is relatively small compared to carbohydrate fermentation, and the contribution of the fat metabolism to gas production is negligible (9). The amount of MGP depends on the molar proportion of VFA produced. Theoretically, the maximum amount of gas is produced when 1 mmol of glucose is fermented to acetic acid and the minimum amount of gas is produced when 1 mmol of glucose is fermented to propionic acid (8).

Details and principles of the techniques for the measurement of gas production have recently been reviewed by Getachew et al. (10) and Theodorou et al. (11). The method for measuring gas production during the incubation of feeding stuff with liquor in vitro was first described by Menke et al. (1). Later automated and computerised gas measurement systems were reported by which fermentation kinetics can also be determined in vitro (12). Theodorou et al. (2) and Davies et al. (13) described a pressure transducer technique and an automated pressure evaluation system, respectively, by which gas accumulation in the headspace of each bottle is measured manually or automatically (respectively) at regular intervals throughout the fermentation using a pressure transducer.

The aim of this study was to determine the MGP, fermentation kinetics and dry matter (DM) loss of different crops in vitro. The effects of particle size on MGP and digestibility were also examined. The potential nutritive value of ground silages, fresh silages, and refusals, rejected feed resulting from 24 h of selective feeding by animals, were estimated using the gas production technique.

Materials and Methods

Silage materials

Silages were prepared from a range of alternative forage crops (barley/pea (*Hordeum vulgare*), clover (*Trifolium hybridum hybridum*), grass (*Lolium multiflorum*), kale (*Brassica oleracea*), lotus (*Nelumbo nucifera*), lucerne (*Medicago sativa* subsp. *varia*) and sainfoin (*Onobrychis montana*) and mown on the dates shown in Table 1. The wilted forage was baled by a precision chop round baler and wrapped with 6 layers of film wrap. The bales were stored until they were fed to sheep, approximately 4 months after.

Half of the silage material from each barley/pea bi-crop harvest and kale were inoculated with fresh culture of *Lactobacillus plantarum* (BioSource Flavors, Inc. Wisconsin, USA) at the rate of 10^6 colony forming units per gram fresh forage (14). Silages were either analysed as fresh samples chopped into 2 cm lengths (fresh silages) or freeze dried for 48 h and ground to pass through a 1 mm dry mesh screen (ground silages) and stored in an airtight plastic container until required for gas production studies. Sheep were fed a silage diet ad libitum to enable

Table 1. The crops that were ensiled for use in this study.

Forages	Stage of maturity	Cutting date	Silage pH	Lactic acid g/kg DM	Ammonia g/kg
Barley/Pea M_1	11 weeks	10 July	4.19	61.32	17.7
Barley/Pea M_1I	11 weeks	10 July	3.79	87.54	18.3
Barley/Pea M_2	14 weeks	4 August	4.63	44.97	5.5
Barley/Pea M_2I	14 weeks	4 August	4.37	68.11	18.7
Clover M_1	6 weeks	8 September	5.34	28.73	95.0
Clover M_2	8 weeks	22 September	5.26	39.42	93.6
Grass	6 weeks	29 July	4.75	76.88	13.3
Kale M_1	14 weeks	8 August	4.05	144.60	102.4
Kale M_1I	14 week	8 August	4.07	150.82	103.6
Kale M_2	17 weeks	26 August	4.06	162.18	85.0
Kale M_2I	17 weeks	26 August	3.99	172.39	88.3
Lotus	8 weeks	22 September	5.14	9.42	31.8
Lucerne M_1	6 weeks	8 September	5.56	35.22	74.5
Lucerne M_2	8 weeks	22 September	5.28	12.18	49.5
Sainfoin	6 weeks	28 July	4.15	57.65	42.9

where I means inoculated with fresh *Lactobacillus plantarum* culture. M_1 and M_2 are stage of maturity and the date of cutting was indicated.

measurement of intake and digestibility, and silage remaining from the previous day's feed was retained as a refusal sample (refusals). Refusals from 10 different samples (barley/pea M_1, barley/pea M_2, clover M_1, clover M_2, kale M_1, kale M_2, lotus, lucerne M_1, lucerne M_2, and sainfoin) were collected, chopped into 2 cm lengths and used for in vitro MGP. A ground grass silage sample was used as a standard over all experiments to account for any rumen fluid differences between experiments so that MGP could be calibrated across all experiments.

Anaerobic medium and chemicals

The medium used in this study was based on that described by Theodorou (15). All chemicals were from Sigma and were of the highest purity routinely available.

Determination of in vitro gas production

The manual PTT described by Theodorou et al. (2) was used to determine the gas production and fermentation kinetics as explained below. A pressure transducer and LED digital readout voltmeter (Bailey and Mackey Ltd., Birmingham, UK) were used to measure the headspace gas pressure of fermenting cultures. Gas pressure in the headspace was read from the display unit and the corresponding volume of gas displaced into a syringe until the gas pressure in the headspace returned to ambient pressure, as indicated by a zero reading on the display unit. Gas measurements were carried out at regular intervals (after 3, 9, 12, 16, 20, 24, 28, 32, 38, 48, 72, 96 and 120 h) during the fermentation period. Bottles were shaken after every reading and were not removed from the water bath (38 ± 1 °C). Both gas pressure and volume in the syringe were recorded in order to correct the possible differences in headspace volumes between bottles.

Rumen fluid source and inoculum

Digesta were taken from a rumen-fistulated sheep fed twice daily on Italian ryegrass (*Lolium multiflorum*) hay, and was immediately transported to the laboratory in vacuum flasks. The digesta were filtered through 3 layers of muslin and the rumen fluid collected in a CO_2-filled flask. The solid residue remaining in the muslin was placed in a blender with some of the strained rumen fluid and homogenised for 30-60 s and strained through the muslin. The resulting rumen fluid was inoculated (10 ml) into each bottle.

VFA analysis

Total VFAs were determined as described by Zhu et al. (16) using a Chrompack model CP9002 gas chromatograph.

Statistical analysis

Differences in total gas production, DM loss and total VFA were analysed using the one-way ANOVA test. The MLP (most likelihood programme) (17) was used to fit curves to experimentally derived gas accumulation profiles using the model of France et al. (18).

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

where $Q = e^{-b}$, $Z = e^{-c}$, *and* $B = e^{bT+c\sqrt{T}}$. Here, y denotes cumulative gas production (ml), t is incubation time (h), A is the asymptotic value for gas pool size (ml), T is the lag time and b (h^{-1}) and c ($h^{-0.5}$) are rate constants.

Results

Gas production from ground silages

Total VFAs, DM loss, total gas production and estimated kinetic parameters (time to produce 50% and 95% of the gas volume and gas pool) for ground silages are presented in tabulated form (Table 2). Kale M_1I produced a significantly higher ($P < 0.05$) gas volume than all the other kale silages and the grass silage. The grass silage produced significantly higher ($P < 0.05$) gas volumes than all the legume silages. Amongst the legumes the total gas volume produced from sainfoin was highest and it did not differ ($P > 0.05$) from that formed by the clover silages. The total gas volume produced by lucerne M_2 was notably lower ($P<0.05$) than that obtained from lucerne M_1. Kale and barley/pea silages produced similar total gas volumes, with the legume silages yielding a remarkably lower ($P < 0.05$) total gas volume.

The fermentation kinetics of kale and legume silages were similar. Barley/pea M_2I took longer to produce 50% and 95% of its final gas pool than did barley/pea M_1I ($P < 0.05$); however, maturity level had no effect on time to produce 50% and 95% final gas pool for other ground silages. Lag time values were similar for all silages (data not shown). The time taken to produce 50% of the final gas pool was longest for barley/pea silages although kale and legume silages behaved similarly. The time taken to

Table 2. Estimated parameters of gas profiles, total VFAs and DM loss of ground silages.

Ground silages	Total VFAs (mMol/l) ± sd	DM loss (g) ± sd	Gas pool (ml) ± sd	Time to produce (h) ± sd	
				50% gas pool	95% gas pool
Barley/Pea M_1I	108.3 ± 2.1^{ab}	0.76 ± 0.01^{ab}	224.0 ± 1.5^{a}	13.6 ± 0.4^{a}	49.1 ± 1.9^{b}
Barley/Pea M_2I	95.3 ± 0.2^{c}	0.70 ± 0.01^{d}	207.2 ± 1.6^{c}	14.4 ± 0.5^{a}	54.6 ± 2.5^{a}
Barley/Pea M_1	104.7 ± 2.4^{b}	0.76 ± 0.02^{ab}	219.1 ± 1.6^{b}	13.7 ± 0.4^{a}	48.5 ± 2.0^{b}
Barley/Pea M_2	103.8 ± 2.2^{b}	0.71 ± 0.02^{cd}	225.3 ± 2.2^{a}	13.7 ± 0.6^{a}	51.0 ± 3.1^{ab}
Clover M_1	95.3 ± 1.2^{c}	0.73 ± 0.03^{bc}	199.7 ± 2.9^{d}	11.1 ± 0.6^{cde}	37.5 ± 3.0^{c}
Clover M_2	87.8 ± 4.9^{d}	0.74 ± 0.01^{bc}	200.6 ± 3.0^{d}	10.4 ± 0.5^{de}	36.6 ± 3.2^{cd}
Grass	95.6 ± 4.2^{c}	0.77 ± 0.01^{a}	218.6 ± 1.9^{b}	12.6 ± 0.4^{b}	45.3 ± 2.3^{b}
Kale M_1I	110.9 ± 2.2^{a}	0.75 ± 0.01^{b}	231.4 ± 8.2^{a}	10.1 ± 0.5^{de}	30.9 ± 2.4^{e}
Kale M_1	97.0 ± 2.1^{c}	0.73 ± 0.01^{c}	216.2 ± 3.4^{b}	10.5 ± 0.6^{de}	31.6 ± 2.5^{de}
Kale M_2I	111.3 ± 1.1^{a}	0.73 ± 0.01^{c}	221.0 ± 2.8^{ab}	9.6 ± 0.4^{e}	30.0 ± 2.1^{e}
Kale M_2	102.1 ± 4.8^{bc}	0.72 ± 0.01^{c}	220.4 ± 3.0^{ab}	10.6 ± 0.5^{de}	32.1 ± 2.3^{de}
Lotus	94.7 ± 2.0^{cd}	0.70 ± 0.01^{d}	201.1 ± 2.6^{d}	10.6 ± 0.5^{de}	36.2 ± 2.7^{cd}
Lucerne M_1	81.4 ± 1.3^{e}	0.63 ± 0.02^{e}	182.8 ± 1.9^{e}	11.3 ± 0.4^{cd}	36.4 ± 2.0^{cd}
Lucerne M_2	84.5 ± 0.5^{d}	0.62 ± 0.01^{e}	175.9 ± 2.2^{f}	12.1 ± 0.5^{bc}	37.9 ± 2.4^{c}
Sainfoin	94.7 ± 2.4^{cd}	0.63 ± 0.01^{e}	209.9 ± 2.9^{c}	9.5 ± 0.5^{e}	35.9 ± 3.2^{cde}

Within a column and forage types, means followed by a different letter differ significantly ($P < 0.05$). I means inoculated with fresh *Lactobacillus plantarum* culture, M means maturity and sd means standard deviation of the mean. Ground grass silage was used as a standard to calibrate MGP.

produce 95% of the final gas pool was longest for barley/pea silages and shortest for kale silages.

Inoculated silages showed higher VFA concentration compared to uninoculated samples, while lucerne silages yielded the lowest VFA level. The lowest DM loss was observed for lucerne and sainfoin silages, while barley/pea M_1 and grass silages showed the highest DM loss. Maturity level had a negative effect on DM loss for barley/pea; however, it was similar for the other silages.

Gas production from fresh silages

Total VFAs, DM loss, total gas production and estimated kinetic parameters (time to produce 50% and 95% of the gas volume and gas pool) for fresh silages are shown in Table 3. The total gas volumes produced by kale (M_1, M_1I and M_2I) silages were significantly higher ($P < 0.05$) than those produced by standard grass silages. However, the total gas volume produced by kale M_2 was similar to that yielded by the grass silage. Kale and barley/pea silages produced similar total gas volumes, with the legume silages generating a significantly lower ($P < 0.05$) total gas volume. The total gas volumes produced by grass, clover M_1 and sainfoin silages were similar and statistically greater ($P < 0.05$) than those derived from clover M_2, lucerne M_1, lucerne M_2 and lotus silages. The gas volumes produced from lucerne M_1 and lucerne M_2 were significantly lower ($P < 0.05$) than those generated by other legume silages. Lag times were similar for all silages (data not shown).

The time taken to produce 50 % and 95% of the final gas pool was longest for barley/pea silages, although kale and legume silages showed similar values.

Kale M_2I produced the highest amount of VFAs, while this amount was lowest for lucerne silages regardless of maturity level. Minimum DM loss was observed for lucerne and sainfoin silages, whilst kale M_1I, grass and kale M_2I showed maximum DM loss.

Gas production from refusals

Total VFAs, DM loss, total gas production and estimated kinetic parameters (time to produce 50% and 95% of the gas volume and gas pool) for refusals are shown in Table 4. The refusals derived from barley/pea M_1 silage produced significantly higher ($P < 0.05$) gas

Table 3. Estimated parameters of gas profiles, total VFAs and DM loss of fresh silages.

Ground silages	Total VFAs (mMol/l) ± sd	DM loss (g) ± sd	Gas pool (ml) ± sd	Time to produce (h) ± sd	
				50% gas pool	95% gas pool
Barley/Pea M_1I	107.0 ± 3.8^{bc}	0.78 ± 0.01^{b}	229.1 ± 2.0^{b}	16.7 ± 0.7^{b}	66.6 ± 4.0^{b}
Barley/Pea M_1	95.7 ± 0.9^{d}	0.73 ± 0.02^{cd}	224.9 ± 1.8^{c}	16.1 ± 0.6^{bc}	60.6 ± 3.0^{b}
Barley/Pea M_2I	03.8 ± 4.6^{c}	0.71 ± 0.01^{d}	222.9 ± 2.8^{c}	21.0 ± 1.5^{a}	85.6 ± 8.1^{a}
Barley/PeaM_2	100.6 ± 3.3^{c}	0.78 ± 0.01^{ab}	225.8 ± 2.7^{b}	22.8 ± 1.7^{a}	89.5 ± 8.1^{a}
Clover M_1	95.6 ± 5.0^{de}	0.74 ± 0.01^{c}	213.2 ± 1.8^{e}	13.9 ± 0.5^{de}	50.5 ± 2.5^{c}
Clover M_2	90.6 ± 4.8^{e}	0.77 ± 0.01^{b}	205.7 ± 1.4^{f}	13.6 ± 0.4^{e}	50.6 ± 2.1^{c}
Grass	95.6 ± 6.3^{de}	0.80 ± 0.01^{a}	218.4 ± 1.9^{d}	12.6 ± 0.5^{f}	45.1 ± 2.4^{cd}
Kale M_1I	111.5 ± 1.4^{b}	0.80 ± 0.01^{a}	240.4 ± 6.3^{a}	13.8 ± 0.6^{de}	45.3 ± 2.7^{de}
Kale M_1	96.8 ± 4.5^{cd}	0.75 ± 0.01^{cd}	230.1 ± 2.9^{b}	13.8 ± 0.6^{de}	44.8 ± 2.8^{de}
Kale M_2I	121.5 ± 2.5^{a}	0.80 ± 0.01^{a}	235.6 ± 2.7^{ab}	13.9 ± 0.6^{de}	45.1 ± 2.6^{de}
Kale M_2	99.8 ± 3.6^{c}	0.72 ± 0.01^{d}	222.0 ± 3.1^{cd}	14.9 ± 0.8^{dc}	48.4 ± 3.3^{cd}
Lotus	93.0 ± 0.1^{e}	0.74 ± 0.01^{c}	200.8 ± 1.0^{f}	11.4 ± 0.2^{g}	42.8 ± 1.4^{e}
Lucerne M_1	74.1 ± 7.8^{f}	0.68 ± 0.02^{e}	180.6 ± 1.3^{g}	13.5 ± 0.4^{ef}	47.2 ± 1.9^{cd}
Lucerne M_2	65.4 ± 2.2^{f}	0.66 ± 0.01^{e}	175.0 ± 1.1^{h}	13.1 ± 0.3^{ef}	47.5 ± 1.7^{cd}
Sainfoin	89.5 ± 7.6^{e}	0.67 ± 0.01^{e}	212.6 ± 1.3^{e}	12.9 ± 0.3^{f}	48.7 ± 1.8^{cd}

Within a column and forage types, means followed by a different letter differ significantly ($P < 0.05$). I means inoculated with fresh *Lactobacillus plantarum* culture, M means maturity and sd means standard deviation of the mean. Ground grass silage was used as a standard to calibrate MGP.

Table 4. Estimated parameters of gas profiles, total VFAs and DM loss of refusal samples.

Ground silages	Total VFAs (mMol/l) ± sd	DM loss (g) ± sd	Gas pool (ml) ± sd	Time to produce (h) ± sd	
				50% gas pool	95% gas pool
Barley/Pea M_1	78.5 ± 5.2^{b}	0.68 ± 0.03^{b}	212.0 ± 1.6^{b}	20.1 ± 0.8^{b}	76.4 ± 3.9^{b}
Barley/Pea M_2	71.7 ± 2.3^{bc}	0.53 ± 0.02^{d}	170.1 ± 1.7^{e}	23.7 ± 1.4^{a}	89.6 ± 6.2^{a}
Clover M_1	72.9 ± 9.3^{bc}	0.65 ± 0.03^{b}	183.9 ± 1.7^{c}	16.5 ± 0.6^{c}	58.6 ± 2.9^{c}
Clover M_2	61.0 ± 4.1^{c}	0.66 ± 0.02^{b}	181.3 ± 1.3^{cd}	16.0 ± 0.5^{c}	58.1 ± 2.5^{c}
Grass	95.6 ± 4.5^{a}	0.78 ± 0.01^{a}	218.4 ± 2.0^{a}	12.6 ± 0.4^{e}	45.1 ± 2.4^{d}
Kale M_1	69.6 ± 6.4^{bc}	0.53 ± 0.03^{d}	177.8 ± 3.1^{d}	19.0 ± 1.3^{b}	61.2 ± 5.3^{c}
Kale M_2	62.1 ± 4.3^{c}	0.47 ± 0.04^{e}	145.9 ± 2.4^{g}	18.4 ± 1.1^{b}	58.4 ± 4.6^{c}
Lotus	68.2 ± 3.4^{c}	0.68 ± 0.02^{b}	183.5 ± 1.2^{c}	14.0 ± 0.4^{d}	53.1 ± 2.2^{c}
Lucerne M_1	60.9 ± 4.1^{c}	0.56 ± 0.04^{cd}	147.5 ± 1.2^{g}	16.3 ± 0.5^{c}	56.2 ± 2.4^{c}
Lucerne M_2	61.0 ± 7.3^{c}	0.56 ± 0.03^{cd}	153.5 ± 1.2^{f}	15.8 ± 0.5^{c}	54.7 ± 2.4^{c}
Sainfoin	74.4 ± 4.3^{b}	0.59 ± 0.02^{c}	181.2 ± 1.2^{cd}	16.2 ± 0.5^{c}	60.7 ± 2.5^{c}

Within a column and forage types, means followed by a different letter differ significantly ($P < 0.05$). M means maturity and sd means standard deviation of the mean. Ground grass silage was used as a standard to calibrate MGP.

volumes than did the other refusal samples. Kale M_2 refusal samples produced the smallest gas volume but this value was not significantly different from lucerne refusals ($P > 0.05$). Kale M_1, clover, lotus and sainfoin refusal samples produced similar total gas volumes. Barley/pea M_2 gave significantly higher ($P < 0.05$) total gas volume than did lucerne and kale M_2 refusals.

The longest lag time was recorded for kale refusal samples (data not shown). The time taken to produce 50% of the final gas pool was longest in the case of barley/pea M_2 refusals and shortest with legume refusals. Legume and kale refusals took similar times to produce 95% of the final gas pool, which were remarkably lower ($P < 0.05$) than the equivalent times noted with barley/pea refusals.

Grass silage yielded more VFA than all refusals while sainfoin, barley/pea clover M_1 and kale M_1 produced higher amounts of VFA within the refusals. Parallel to VFA and total gas pool, DM loss occurred in the highest level for grass silage compared to refusals.

Relationship between total VFAs formation, DM loss and total gas volume

For dried and ground silages the correlation between total gas volume and total VFA concentration was remarkably significant ($P < 0.01$), $r^2 = 0.74$, as was the correlation between DM loss and total gas volume ($P < 0.05$), $r^2 = 0.53$. However, no significant correlation was found between DM loss and total VFAs for the dried ground silages, $r^2 = 0.41$. For the fresh silages the correlation between total gas volume and total VFA concentration was significant ($P < 0.01$), $r^2 = 0.86$, as was the correlation between total gas volume and DM loss ($P < 0.05$), $r^2 = 0.52$. The correlation between total VFA concentration and DM loss was also statistically significant ($P < 0.05$), $r^2 = 0.57$. For the refusal samples the correlation between total gas volume and total VFA concentration was statistically significant ($P < 0.01$), $r^2 = 0.72$. The correlation between total gas volume and DM loss for the refusal samples was statistically significant ($P < 0.01$), $r^2 = 0.74$, although no significant relationship was recorded between total VFA concentration and DM loss, $r^2 = 0.46$.

The mean values of the parameters (total gas volume, total VFAs and in vitro DM loss) were similar for ground and fresh silages. However, the mean values of these parameters for refusals were lower than those for the fresh and ground silage samples. All silages (ground or fresh) and refusal samples produced VFAs in similar molar proportions with no significant differences ($P > 0.05$) (data not shown). The molar proportion of acetate was between 55% and 61%, that of propionate was between 23% and 31%, that of butyrate ranged from 9% to 13% and that of valerate ranged from 0.030% to 0.069%.

Discussion

Total gas production from the fermentation of kale and barley/pea silages was higher than that from legume silages. This could be due to the fact that the legumes are a relatively rich source of protein, which yields less gas than carbohydrates (19,20). A higher total gas production was determined from the fermentation of barley/pea silage of maturity 1 than of maturity 2 and this could be explained by chemical structural changes of plant tissue (20).

Nagadi et al. (21) indicated that microbial gas production from ground and fresh forage was similar. In current study, only small differences in total gas production between ground and fresh silages were observed. These small differences in total gas volume could be a result of different leaf to stem ratios with particularly difficult-to-chop fresh silage samples such as kale. On the other hand fresh and ground silages took similar times to produce 95% of their final gas yields but different times to produce 50% of the gas pool. This could be explained by the fact that ground silage material can supply more surfaces for attachment of rumen microflora, particularly for the rumen bacteria.

A strong relationship was found between ground and fresh silages in the time taken to produce 95% of the final gas pool, and the lag time. However, the relationship between these 2 types of pre-treatment was poor in respect of the time taken to produce 50% of the final gas pool. Different chemical compositions of the substrates helped to explain the different lengths of their fermentation periods. For example, free glucose and sucrose are fermented more rapidly than starch (22). This could also be explained by ground silage materials supplying more surfaces for the attachment of rumen microorganisms (23).

The easily fermentable carbohydrate contents of kale and legume are similar but the structural carbohydrate

content of kale silages is higher than that of legume silages (20). Therefore, kale and legume silages took similar times to produce 50% of the final gas pools, but kale silages produced 95% of their gas pool more rapidly than did the legume silages. The times taken to produce 50% and 95% of the final gas pool in the case of barley/pea silages differed according to the maturity of the plant material as M_2 took longer than M_1. This can be explained by an increased relative content of protein in the more mature barley/pea silage, which can be readily biodegraded by rumen microorganisms.

The rumen fluid inoculum was obtained from sheep on a diet consisting of high Italian ryegrass. The lag phase could have been shortened if the sheep had been fed a diet consisting of a mixture of all the silages used in these experiments prior to the use of its rumen digesta as inoculum. Another way of reducing the lag phase would have been to allow the rumen digesta to become partially adapted to the feed of interest before being used as inoculum in these experiments. A similar method has previously been successfully used by other researchers to reduce the lag phase (24).

Refusal samples might be expected to be lower in nutritive value than the whole feed due to the sheep having selected the more nourishing parts. The results of this study illustrate that gas production profiles for refusals were lower than those for silages, and the time taken to produce 50% and 95% of the final gas pool was longer. A low leaf to stem ratio in the refusals would increase the time period required to produce 50% of the gas pool. Although the lower digestibility of the refusals was expected, the results showed that they could still be used as feed-stuff since rumen microflora is able to degrade the cell walls of refused plants by sheep.

The current study showed similarities with the reports of Beuvink and Kogut (25) and Mertens (26) in that 50% of the final gas production occurred between 10 and 15 h for ground silages, and between 15 and 20 h for fresh silages. The times taken to produce 50% and 95% of the final gas pools were longer with refusal samples than with fresh or ground silages.

A significant relationship was observed between total VFA concentration and total gas produced for ground silages, fresh silages and refusal samples. Furthermore, the correlation of total gas volumes with VFA concentrations was better than the correlation with DM loss and these results were in agreement with the findings of Getachew et al. (9). Doanne et al. (27) obtained a similar result, namely that the relationship between gas production and VFA production was linear with a significant correlation.

The maturity of the plant prior to ensiling affects the DM digestibility of the resulting silages. The degradation of the more mature material was lower in the case of both kale and barley/pea silages. This was very likely due to the more mature plants having more structural carbohydrate than rapidly fermentable carbohydrate and a higher proportion of protein. Crovetto et al. (28) found similar effects to those reported here, reporting that increasing the stage of maturity significantly reduced the DM digestibility. Mbwile and Uden (29) also showed that organic matter digestibility was affected by the maturity of the plant and harvesting season.

In the case of kale and barley/pea silages, the molar proportion of propionate was higher when the less mature material (M_1) was digested, the reason being that the content of rapidly degradable carbohydrate decreases as the maturity of the kale and barley/pea silage material increases. Butyrate production is chiefly the outcome of protein degradation and all of the silages produced similar molar proportions of butyrate. Barley/pea M_2 produced a higher molar proportion of butyrate than barley/pea silage (M_1), which can be explained by the increase in maturity being associated with enhanced protein content in the barley/pea substrate (20).

However, further research comparing in vivo and in vitro digestibility is required to expand these findings, and to increase our confidence in the accuracy of in vitro methods to estimate the digestibility of a range of forage offered to and the forage refused by animals.

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