Methane production by anaerobic digestion of tall fescue samples pre- and post-ensiling, prepared by thermal or freeze drying

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Abstract: This study investigated the effects of thermal (40ºC) and freeze drying on the chemical composition and specific CH_4 yield of tall fescue, pre- and post-ensiling and at three sequential harvest dates in the primary growth. Tall fescue samples pre- and post-ensiling were either thermally (40ºC) or freeze dried prior to milling. Dried, milled samples were used to determine herbage chemical composition and specific CH_4 yield in a small-scale (160 mL) high-throughput batch digestion test. A relatively small increase in specific CH_4 yield was observed for herbages post- compared with pre-ensiling (237 and 249 L CH_4/kg VS_{added} for herbage pre- and post-ensiling, respectively) and this may reflect the presence of quantities of fermentation products in the dried silage samples. Compared with freeze drying, thermal drying resulted in a small decrease in specific CH_4 yield in batch digestion tests (11, -22 and -17 L CH_4/kg VS_{added} for Harvests 1, 2 and 3, respectively) and this reflected the small changes in herbage chemical composition due to drying method. In general, however, the impact of drying method on herbage chemical composition and specific CH_4 yield was similar for herbages pre- and post-ensiling. More importantly, drying method had little effect on the relative ranking of samples across harvest dates and for samples pre- and post-ensiling.

Keywords: grass, grass silage, thermal drying, freeze drying, anaerobic digestion, biogas

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1 Introduction

Grass is an excellent feedstock for biogas production (Murphy et al., 2011) and will be an important feedstock for on-farm biogas production in much of north west Europe (McEniry et al., 2013). In order to estimate potential biogas yields from large numbers of contrasting grass and grass silage samples the use of small-scale batch digestion systems is necessary. Although the CH_4 potential of grass feedstocks can be obtained from fresh samples, assays are often conducted on samples that have been dried in heated ovens with forced air ventilation and milled through a screen with 1 mm apertures (Lowman et al., 2002). This approach not only enables sample preservation (Van Soest, 1994), but facilitates the processing of a relatively large representative sample of undried herbage to provide a smaller representative sub-sample (< 1 g dried, milled sample in some cases) for subsequent analyses (Purcell et al., 2011). Most small-scale batch digestion systems, including the Hohenheim Biogas Yield Test (Helffrich and Oechsner, 2003) and the majority of international systems for formulating ruminant diets (e.g. the National Research Council, Institut National de la Recherche Agronomique), rely on data determined from thermally dried samples. Typically, samples are dried at temperatures between 40°C and 70°C.

Thermal drying, however, is capable of changing the chemical composition of a feedstock and these changes may impact on feedstock specific CH_4 yield (i.e. L CH_4/kg volatile solids (VS) added) in batch digestion tests. For example, continued activity by plant enzymes during thermal drying at low temperatures can result in organic matter losses (Smith et al., 1973) and this was

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demonstrated by Pelletier et al. (2010) who reported a decrease in total non-structural carbohydrates in grass and alfalfa samples during thermal drying. In contrast, drying at high temperatures can result in the production of 'Maillard' products (Van Soest, 1994) and this was demonstrated by Alomar et al. (2003) and Parissi et al. (2005) who reported an apparent increase in neutral detergent fibre (NDF) and acid detergent lignin concentrations. Thus, freeze drying is often the preferred system for drying grass samples in order to avoid these problems (Smith et al., 1973; Pelletier et al., 2010).

Additional difficulties are encountered when evaluating grass silage samples. This results from the loss of volatile compounds such as fermentation acids and alcohols during both thermal and freeze drying, with Porter and Murray (2001) reporting volatility coefficients of 0.09, 0.55 and 0.99 for lactic acid, volatile fatty acids and alcohols when thermal drying at 60°C, respectively. Subsequent biogas yield values from dried silage samples can consequently be underestimated and may require a correction (Weisbach and Strubelt, 2008). In many cases, however, a full chemical analysis of the fermentation products in silage and an appropriate correction equation are not readily available.

Although freeze drying is considered as the method of reference for drying unfermented feedstocks, thermal drying is more convenient and in most cases is the method of choice. However, grass is usually harvested and stored as silage to ensure a constant supply of feedstock to a biogas facility. Consequently, the impact of drying method needs to be considered for herbages pre- and post-ensiling. Thus, this study investigated the effects of thermal (40ºC) and freeze drying (−55°C for 72 h) on the chemical composition and specific CH_4 yield of tall fescue, pre- and post-ensiling and at three sequential harvest dates in the primary growth. Methane production was determined for dried, milled samples in a small-scale (160 mL) high-throughput batch digestion test.

2 Materials and methods

2.1 Approach

Tall fescue samples pre- and post-ensiling were either thermally (40ºC) or freeze dried (−55°C for 72 h) prior to milling. Dried, milled samples were used to determine herbage chemical composition and specific CH_4 yield in batch digestion tests.

2.2 Harvest and ensiling

Tall fescue (*Festuca arundinacea* Schreb var. Fuego) was grown in field plots (20 m^2) within three replicate blocks at Grange (53°52′N, 06° 66′W). The plots were managed under a high fertiliser nitrogen input of 125 kg N/ha (applied in mid-March) and separate plots were harvested at three dates (12 May, 9 June and 7 July 2011; Harvests 1 to 3, respectively; n = 9 plots) in the primary growth, as described previously by King et al. (2012). At each harvest date, appropriate plots were harvested to a 6 cm stubble height using a Haldrup forage plot harvester (J. Haldrup, Løgstor, Denmark). The herbage was passed through a precision-chop harvester (Pottinger, Mex VI, Grieskirchen, Austria) and representative 6 kg samples were ensiled in laboratory silos for 100 days (O'Kiely and Wilson, 1991). A representative sample of each herbage pre- and post-ensiling was taken and stored at −18ºC (McEniry et al., 2006).

2.2 Herbage preparation - drying

When required for analysis, individual grass and silage samples were thawed at 4°C for 24 h, bowl-chopped (Muller MKT 204 Special Food Processor, Saarbrücken, Germany) and separately mixed. Subsequently, representative subsamples (200 g) of each herbage were either thermally dried in a ventilated oven with forced air circulation at 40°C for 48 h or freeze dried at −55°C for 72 h (Scanvac Coolsafe, model no. 55-4, Labogene, Denmark) prior to milling (Wiley mill, 1 mm aperture screen). Thermal drying at this temperature is the standard procedure when preparing forage samples for chemical analysis at Teagasc Grange (Purcell et al., 2011).

2.3 Herbage chemical composition

Dried, milled samples were used for the determination of total solids digestibility (TSD), NDF, acid detergent fibre (ADF), crude protein (CP), ash and water soluble carbohydrate (WSC) concentrations. Total solids digestibility was determined by the method of Tilley and Terry (1963), but with the final residue being isolated by filtration (Whatman GF/A 55 mm, pore size 1.6 mm;

Whatman International, Maidstone, UK) rather than centrifugation. Neutral detergent fibre (assayed with a heat stable alpha-amylase and sodium sulphite) and ADF were determined by the method of Van Soest (1963) using ANKOM filter bag technology and expressed exclusive of residual ash. The ash content was determined by complete combustion in a muffle furnace at 550°C for 5 h and this allowed for subsequent calculation of herbage VS (equivalent to organic matter) concentration (VS = TS – ash). Crude protein concentration (N × 6.25) was determined using a LECO FP428N analyser (Leco Instruments, MI, USA) based on AOAC method 990–03 (AOAC, 1990), while the concentration of WSC was determined according to the anthrone method (Thomas, 1977) using an Autoanalyser 3 (Bran and Leubbe GmbH, Norderstedt, Germany).

Using silage samples taken prior to drying, the pH was determined from an aqueous extract using a pH electrode (Orion SA720 pH meter). Further aqueous extracts were used for analysis of lactic acid (LA), volatile fatty acids (VFA) and ethanol, as previously described (McEniry et al., 2006), while the concentration of ammonia-N (NH_3-N) was determined using an enzymatic-UV kit (Thermo Electron Infinity Ammonia Liquid Stable reagent) on an Olympus AU400 (Olympus UK Ltd., Hertfordshire) clinical analyser.

Representative herbage samples pre- and post-ensiling were also dried at 98°C and 85°C, respectively, for 16 h in an oven with forced air circulation to estimate total solids (TS) concentration and silage samples were corrected for the loss of volatiles by the equation of Porter and Murray (2001). The pH and the TS and VS concentration of the sludge inoculum were also determined using methods described above.

2.4 Batch digestion tests

The CH_4 produced from each grass and silage sample (dried, milled) was determined in duplicate in 160 mL batch digestion tests, in accordance with VDI 4630 (2006) and as described previously by McEniry et al. (2013). Briefly, inoculum and substrate were added to 160 mL incubation bottles at a VS inoculum to substrate ratio of 2:1 and at a final VS concentration of 10 g/kg. The inoculum (pH = 7.98; 4 g TS/kg, 2 g VS/kg) was obtained from an anaerobic digester using cattle manure at the Agri-Food and Biosciences Institute in Hillsborough, Northern Ireland. Micro- [$MgSO_4.7H_2O$, 5 mg/L; H_3BO_3, 0.3 mg/L; $ZnCl_2$, 0.1 mg/L; $NiCl_2.6H_2O$, 0.75 mg/L; $MnCl_2.4H_2O$, 1 mg/L; $CuCl_2.2H_2O$, 0.1 mg/L; $CoCl_2.6H_2O$, 1.5 mg/L; $Na_2SeO_3.5H_2O$, 0.02 mg/L; $Al_2(SO_4)_3.18H_2O$, 0.1 mg/L; $(NH_4)6Mo_7O_{24}.4H_2O$, 0.1 mg/L] and macro- [NH_4HCO_3, 0.4 g/L; $KHCO_3$, 0.4 g/L; $NaHCO_3$, 0.4 g/L] mineral solutions (Gonzalez-Gil et al., 2001) were added to each bottle to ensure that nutrient conditions were not limiting. Distilled water was then added to each bottle to adjust the final volume of inoculum and substrate in the incubation bottle to 70 mL.

In order to quantify the CH_4 produced by the inoculum, six replicate bottles with no substrate (i.e. blanks) were incubated under the same conditions. In addition, six replicate cellulose (Sigma, 22184) samples were used as a positive control to provide a standardised rating of the biological activity of the inoculum. The final pH in each bottle was adjusted to 7.2 with 1 M hydrochloric acid before the contents were flushed with N_2 gas for 1 minute and sealed with butyl rubber stoppers and aluminium crimp seals. Bottles were incubated at 38°C for 36 days and agitated daily.

Using a detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK), the gas headspace pressure inside each bottle was recorded after 2, 5, 8, 13, 19, 26 and 36 days incubation. The total amount of biogas produced was estimated using the following equation: Biogas production (mL) = (vh/Pa) × Pt; where vh is the headspace volume (mL), Pa is the atmospheric pressure (hPa) and Pt is the gas headspace pressure (hPa). Following the determination of biogas volume, a 0.8 mL sample of gas was used to determine CH_4 concentration by gas chromatography using a Shimadzu GC-2014 with a flame ionisation detector equipped with a glass column (2.1 m × 5 mm × 3.2 mm packed with molecular sieve 5A 60/80 mesh). Temperatures were 120ºC in the column, 150ºC in the injector and 170ºC in the detector, and N_2 was used as the carrier gas (Lovett et al., 2004; Bodas et al., 2008). Triplicate gas standards of known CH_4 concentration

were used to construct a calibration curve. After each sampling, the gas pressure inside each bottle was released.

Evaluation of these data included the following steps (VDI 4630, 2006): (a) headspace correction of the biogas values on day 2, as inert gas in the headspace at the beginning (day 0) of the batch digestion test causes a dilution of the biogas components, (b) subtraction of the volume of CH_4 produced by the inoculum (i.e. blank) from the volume of CH_4 produced in the batch digestion test with substrate and inoculum and (c) normalising the CH_4 volume to standard temperature and pressure conditions (i.e. dry gas, 273 Kelvin, 1013 hPa). The specific CH_4 yield (L CH_4/kg VS_{added}) was calculated as the cumulative sum of the CH_4 volume produced over the 36 day incubation period relative to the substrate VS concentration added to the test.

2.5 Statistical analyses

Means and standard deviations (s.d.) were calculated for silage fermentation variables determined for samples prior to drying and are presented in Table 1.

Table 1 Mean (s.d.) silage total solids concentration (TS) and fermentation variables (g/kg TS, unless indicated otherwise; except pH) prior to drying

Harvest[1]	TS/g kg^{-1}	Silage fermentation variables[2]								
		pH	LA	AA	PA	BA	EtOH	FP	LA/FP/g g^{-1}	NH_3-N/g kg^{-1} N)
1	167 (3.8)	4.24 (0.095)	57 (28.3)	41 (5.8)	6.2 (0.89)	1.9 (3.28)	38 (6.0)	144 (32.4)	0.38 (0.13)	62 (30.9)
2	209 (18.5)	3.93 (0.170)	74 (10.4)	20 (9.1)	0.8 (1.46)	0.2 (0.42)	15 (4.7)	110 (9.3)	0.67 (0.11)	64 (24.6)
3	218 (9.3)	3.76 (0.295)	66 (27.3)	28 (11.3)	0.9 (0.87)	0 (0)	23 (9.3)	118 (10.4)	0.55 (0.21)	63 (2.6)

Note: [1] Harvest 1 = 12 May, Harvest 2 = 9 June, Harvest 3 = 7 July 2011

[2] LA = lactic acid, AA = acetic acid, PA = propionic acid, BA = butyric acid, EtOH = ethanol, FP = total fermentation products (LA + AA + PA + BA + EtOH), LA/FP = lactic acid as a proportion of total fermentation products, NH_3-N = ammonia-N

Methane production was determined in duplicate batch digestion tests for each of the three replicate samples per treatment. These duplicate values were averaged to give a single value for each sample for subsequent statistical analysis. Herbage chemical composition and specific CH_4 yield data were analysed as a split-plot design using the MIXED procedure of SAS, Version 9.1.2. The structure used harvest date as the main plot, with a 2 (ensiling; pre- or post-ensiling) × 2 (drying method; thermal or freeze drying) factorial arrangement of treatments within the sub-plot, and with the effect of replicate block being accounted for within the main plot. The Tukey adjustment for multiple comparisons was used in testing for differences between means.

The CH_4 production at each individual sampling day (2, 5, 8, 13, 19, 26 and 36) over the 36 day incubation period (i.e. CH_4 production over time) was analysed as described above, but with the repeated measures effect of sampling day also being accounted for.

3 Results

3.1 Batch digestion tests

On average, 0.75 of total CH_4 production over the 36 day incubation period was produced by day 13 of the batch digestion test. The specific CH_4 yield for the herbages pre- and post-ensiling varied from 216 to 273 L CH_4/kg VS_{added} (Table 2). The mean (s.d.) specific CH_4 yield for the cellulose control was 256 (9.4) L CH_4/kg VS_{added}.

3.2 Herbage chemical composition and specific CH_4 yield

3.2.1 Harvest date

Grass TS concentration was 184, 213 and 217 g/kg for Harvests 1, 2 and 3, respectively. Herbage TSD decreased (P<0.001) while NDF concentration increased (P<0.001) with advancing harvest date (Table 2). On average, ADF concentration was lowest (P<0.001) for herbage from Harvest 1 compared with the later harvest dates, while the opposite (P<0.001) was the case for CP and WSC concentrations. The specific CH_4 yield was lower (P<0.05) for the Harvests 2 and 3 compared with the Harvest 1 herbage (Table 2), and these herbages also had an apparent slower (P<0.001) rate of digestion (Figure 1).

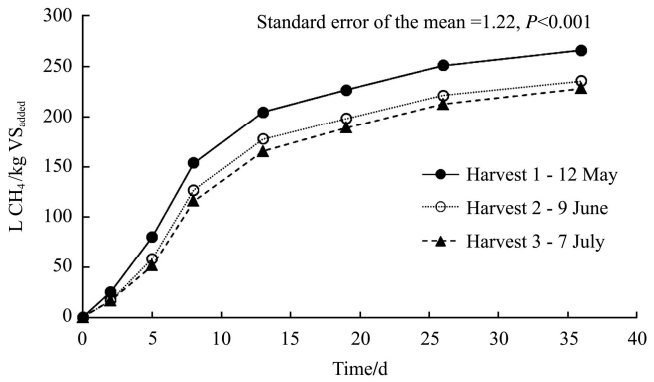

Figure 1 Effect of harvest date on cumulative CH_4 production over the course of the 36 day incubation period in batch digestion tests (averaged across ensiling and drying treatments)

3.2.2 Ensiling

On average, ensiling resulted in a decrease in herbage TSD (P<0.01) and WSC concentration (P<0.001) and an increase in ADF (P<0.001), CP (P<0.01) and ash (P<0.01) concentrations and specific CH_4 yield (P<0.05) (Table 2).

3.2.3 Drying method

Thermal drying resulted in a higher (P<0.001) NDF concentration and lower herbage CP (P<0.001) and WSC (P<0.001) concentrations, and a lower specific CH_4 yield (P<0.05) compared to freeze drying (Table 2).

3.2.4 Harvest date x ensiling

Although ensiling resulted in a numerical increase in ADF concentration at each harvest date, this difference was only significant (P<0.05) for the Harvest 1 herbage. Similarly, ensiling resulted in an increase (P<0.05) in ash concentration for the Harvest 1 herbage only. In addition, the decrease in WSC with ensiling was higher (P<0.001) for the Harvest 1 compared with the later harvest dates.

Table 2 Effect of harvest date, ensiling and drying method on the chemical composition (g/kg TS, unless indicated otherwise) and specific CH_4 yield (L CH_4/kg VS_{added}) of tall fescue

Harvest date[1]	Ensiling	Drying method[2]	Chemical composition[3]						Specific CH_4 yield
			TSD/g kg^{-1}	NDF	ADF	CP	Ash	WSC	
1	Grass	Thermal	788	529	267	152	86	161	268
1	Grass	Freeze	801	471	270	164	87	184	257
1	Silage	Thermal	784	536	321	167	104	4	273
1	Silage	Freeze	770	534	329	168	95	7	265
2	Grass	Thermal	680	623	372	112	90	92	219
2	Grass	Freeze	679	602	357	121	89	100	232
2	Silage	Thermal	641	642	376	117	95	8	231
2	Silage	Freeze	643	570	358	131	97	10	261
3	Grass	Thermal	582	653	381	97	84	88	216
3	Grass	Freeze	528	610	365	117	86	105	230
3	Silage	Thermal	530	667	397	106	84	10	223
3	Silage	Freeze	510	625	382	114	83	11	243
Standard error of the mean									
Harvest (H)			11.9	8.1	5.2	3.6	2.6	2.2	4.8
Ensiling (E)			7.9	5.9	4.0	2.4	1.7	1.5	3.3
Drying method (D)			7.9	5.9	4.0	2.4	1.7	1.5	3.3
H × E × D			16.6	13.5	9.5	5.0	3.6	3.3	7.2
Levels of significance[4]									
H			***	***	***	***	NS	***	*
E			**	NS	***	**	**	***	*
D			NS	***	NS	***	NS	***	*
H × E			NS	NS	*	NS	*	***	NS
H × D			NS	NS	NS	NS	NS	NS	**
E × D			NS	NS	NS	NS	NS	***	NS
H × E × D			NS	*	NS	NS	NS	NS	NS

Note: [1] Harvest 1 = 12 May, Harvest 2 = 9 June, Harvest 3 = 7 July;

[2] Thermal = thermally dried in a ventilated oven with forced air circulation at 40ºC for 48 h, freeze = freeze dried at -55ºC for 72 h;

[3] TSD = total solids digestibility, NDF = neutral detergent fibre, ADF = acid detergent fibre, CP = crude protein, WSC = water soluble carbohydrates;

[4] * = P<0.05, ** = P<0.01, *** = P<0.001, NS = not significant.

3.2.5 Harvest date x drying method

Compared with freeze drying, the thermal drying treatment resulted in a lower specific CH_4 yield (P<0.01) for the Harvests 2 and 3 herbages, while no difference (P>0.05) was observed for the Harvest 1 herbage.

3.2.6 Ensiling x drying method

The decrease in WSC concentration, as a result of thermal compared with freeze drying, was higher (P<0.001) for the herbages pre-ensiling compared to the herbage post-ensiling.

3.2.7 Harvest date x ensiling x drying method

With the exception of the Harvest 1 silages, thermal drying resulted in a higher (P<0.001) NDF concentration compared to freeze drying. There were no further three-way interactions (P>0.05) for any of the variables measured.

4 Discussion

4.1 Batch digestion tests

The specific CH_4 yield varied from 216 to 273 L CH_4/kg VS_{added} in this study, which was at the lower end of the wide range of values reported in the literature for grass and grass silage samples (198 to 467 L CH_4/kg VS) (Prochnow et al., 2009; Murphy et al., 2011). The lower than expected CH_4 yield from the cellulose control (0.68 of expected yield; VDI 4630, (2006)) suggests that the activity of the inoculum was relatively low.

4.2 Harvest date

The use of a tall fescue crop harvested at a series of advancing stages of maturity provided grass spanning a range of chemical and physical attributes, and strengthens the subsequent comparisons of the effects of thermal (40ºC) and freeze drying on the chemical composition and specific CH_4 yield of tall fescue, pre- and post-ensiling.

Delaying the harvest date had a negative influence on specific CH_4 yield in the current study and this is in accordance with Amon et al. (2007) and Lehtomaki et al. (2008). This reflects the general decrease in the plant leaf to stem ratio and the increasing cell wall content (i.e. NDF and ADF) within the stems as the plant matures (Buxton, 1996; Stefanon et al., 1996). Since this process is accompanied by increasing lignification within the cell wall fraction, there was an overall decrease in TSD and specific CH_4 yield. The slower apparent digestion rate of this more mature (i.e. fibrous) material would result in a longer retention time in the digester if the CH_4 yield (per unit VS incubated) is to be maintained. Advancing harvest date was also characterised by a decrease in herbage CP and WSC concentrations, in accordance with Mason and Lachance (1983) and Tremblay et al. (2005), respectively. This can also be attributed to the declining cell content to cell wall ratio (Buxton, 1996).

4.3 Ensiling

For the silage samples taken prior to drying, BA concentration was less than 5 g/kg TS and ammonia-N concentration was less than 65 g/kg N for all silages indicating successful preservation (Haigh and Parker, 1985). The lower concentration of LA in total fermentation products for the Harvest 1 silages possibly reflects a more heterofermentative fermentation in this silage, with values of 41, 20 and 28 g/kg TS and 38, 15 and 23 g/kg TS for AA and ethanol, for Harvests 1, 2 and 3, respectively. The low LA/FP and the high ethanol concentration for Harvest 1 silage may also reflect yeast fermentation of sugars which were surplus to the requirement for lactic acid to provide a sufficient reduction in pH (Chamberlain, 1989).

Despite the small increase in ADF concentration and decrease in TSD with ensiling, a relatively small increase in specific CH_4 yield (237 and 249 L CH_4/kg VS_{added} for herbage pre- and post-ensiling, respectively) was observed in batch digestion tests. This is in contrast to McEniry et al. (2013) who reported a positive correlation (r = 0.8717) between specific CH_4 yield and increasing herbage TSD.

The specific CH_4 yield of some silages has been reported to be higher than pre-ensiled herbage due to the formation of fermentation products (e.g. ethanol, 1,2-propanediol) with a higher potential CH_4 yield than the original fermentation substrates (Pakarinen et al., 2008; Herrmann et al., 2011). It has also been suggested that ensiling increases the rate of CH_4 formation (Heiermann et al., 2002) as some of the fermentation products produced act as precursors to CH_4 formation.

However, volatile compounds such as fermentation acids and alcohols would have been lost during thermal (Weisbach and Strubelt, 2008; Porter and Murray, 2001) and freeze drying (Abascal et al., 2005; CAB, 1961) in this study. Although the volatility of the silage fermentation products at 40ºC would have been considerably lower than that reported by Porter and Murray (2001) and Weisbach and Strubelt (2008) when drying from 60ºC to 100ºC, some partial losses would still have occurred. Volatile compounds would also have been lost during freeze drying, but little information is available on the volatility of silage fermentation products during freeze drying. The presence of quantities of these fermentation products in the dried silage may explain the small differences in specific CH_4 yield for herbages pre- and post-ensiling. Similarly, considering the partial losses of these volatile compounds during drying, it could also be postulated that the specific CH_4 yield of these silage samples could have been underestimated in this study.

The relative proportions of ADF, CP and ash increased during ensiling, reflecting the loss of water soluble organic matter during fermentation and through effluent production (McDonald et al., 1991). This was particularly evident for the Harvest 1 herbages, where effluent production was higher than at later harvest dates (200, 116 and 136 g effluent/kg fresh herbage ensiled for Harvests 1, 2 and 3, respectively). This contributed to the small decrease in TSD (30 g/kg). The reduction in WSC concentration during ensiling indicates its substantial use as a substrate for fermentation. The magnitude of decrease in WSC was also greater for the Harvest 1 herbage reflecting the more extensive fermentation (144, 110 and 118 g/kg TS fermentation products for Harvests 1, 2 and 3 silages, respectively) in this herbage during ensiling.

In general, for the dried, milled samples in this study ensiling had only a moderate effect on specific CH_4 yield (caused a proportionately 0.05 increase) and had little effect on the relative ranking of the silage samples compared to herbage samples pre-ensiling.

4.4 Drying method

To the best of the author's knowledge, no studies have compared effects of thermal or freeze drying on CH_4 yield of herbages pre-and post-ensiling in batch digestion tests. However, a number of authors have reported that drying method has little or no effect on gas production using the *in-vitro* gas production technique (Purcell et al. 2011; Parissi et al., 2005; Lowman et al., 2002), a short-term (<120 h) batch test with rumen microorganisms.

Compared with freeze drying, thermal drying resulted in a small decrease in specific CH_4 yield in batch digestion tests, but this was only evident for the later harvest dates (11, -22 and -17 L CH_4/kg VS_{added} for Harvests 1, 2 and 3, respectively). Although, an explanation for this harvest date effect is not apparent, the small difference in specific CH_4 yield between the herbages prepared by thermal or freeze drying was generally reflected in the small-scale differences in the chemical composition of these herbages as a result of drying method. Furthermore, there was no difference in this trend for herbages pre- and post-ensiling, possibly reflecting a similar extent of loss of WSC and volatile compounds (e.g. fermentation acids and alcohols) during drying for the herbages pre- and post-ensiling, respectively.

The higher herbage NDF concentration with thermal compared to freeze drying is consistent with Alomar et al. (2003) and Parissi et al. (2005) who reported increases in NDF concentration of 38 and 45 g/kg for thermal drying at 60°C, respectively. This has been attributed to a reduction in protein solubility during thermal drying and a subsequent increase in neutral detergent insoluble nitrogen (Deinum et al., 1994). Van Soest (1994) described two forms of protein condensation as affected by thermal drying: (a) the formation of indigestible protein-carbohydrate complexes (i.e. Maillard products) at higher temperatures and (b) protein denaturation and the formation of insoluble (in neutral detergent) but still digestible compounds at medium temperatures. This is further supported by the relatively modest effect of drying method on ADF concentration, in agreement with Alomar et al. (2003). This can result in the measurement of lower concentrations of sugars and true protein than those present in the fresh sample, together with higher apparent

values for plant cell walls (Deinum et al., 1994; Pelletier et al., 2010). In addition, continued plant and microbial respiration during the early stages of thermal drying may also have contributed to the loss of WSC. The greater decrease in WSC concentration for the herbage pre-ensiling compared with the herbage post-ensiling, as a result of thermal drying, reflects the higher WSC concentration in the herbage pre-ensiling (Lopez et al., 1995).

Although a reduced TSD could be expected as NDF concentration increased, the trend of a lower NDF concentration in the freeze dried samples did not result in a higher herbage digestibility. This is in agreement with Tilley and Terry (1963) who reported that forage samples have similar *in-vitro* rumen digestibility whether plant material is thermal or freeze dried at 40ºC or 100 ºC.

5 Conclusions

The relatively small-scale effects of ensiling (with the exception of the conversion of WSC to fermentation products) and drying method on herbage chemical composition were reflected in the relatively small differences in specific CH_4 yield in batch digestion tests. The impact of drying method on herbage chemical composition and specific CH_4 yield was similar for herbages pre- and post-ensiling. Although freeze drying gave a higher specific CH_4 yield in some instances, the relatively small differences between the drying methods and more importantly the absence of a change in the ranking of the treatments (across harvest dates and for samples pre- and post-ensiling), suggests that thermal drying will continue to be the method of choice for sample preparation for batch digestion tests.

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