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In vitro rumen methane output of perennial ryegrass samples prepared by freeze drying or thermal drying (40 °C)

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ABSTRACT

In many *in vitro* studies feeds are dried in heated ovens with forced air ventilation and milled through a screen with 1 mm apertures to obtain a physically representative small sample. Drying methods involving heat have been shown to change the chemical composition of feeds, and it is possible that changes in the chemical composition during thermal drying could affect *in vitro* rumen CH_4 output measured on the samples. Hence, samples are often dried by freeze drying. The objective of this study was to compare effects of freeze drying at −55 °C for 72 h *versus* thermal drying at 40 °C for 48 h on the chemical composition and CH_4 output of perennial ryegrass samples collected at 5 stages of primary growth (*i.e.*, 12 May, 26 May, 9 June, 23 June, 7 July) using an *in vitro* rumen gas production technique. The magnitude of the changes in chemical composition due to drying method was small and not biologically important, and drying method had no effect on CH_4 output. With advancing maturity at harvest, digestibility of the herbage declined. Methane output/g dry matter (DM) incubated decreased linearly while CH_4 output/g DM digested increased linearly with advancing grass maturity. Thermal drying on a range of grass samples had a similar effect to freeze drying on *in vitro* rumen CH_4 output, reflecting the small degree of change in chemical composition.

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1. Introduction

In many studies, the assays undertaken to characterise feeds are conducted on samples that have been dried in heated ovens with forced air ventilation and milled through a screen with 1 mm apertures (Lovett et al., 2004, 2006; García-González et al., 2008; Longo et al., 2006). This approach facilitates a relatively large representative sample (*i.e.*, 100 g or more) of undried herbage being processed to permit a physically representative but small sub sample (often <1 g dried, milled herbage) being

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; aDMD, apparent DM disappearance; ADIN, acid detergent insoluble N; A:P, acetic acid to propionic acid; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; NGGR, non-glucogenic to glucogenic ratio; OMD, *in vitro* organic matter digestibility; RF, rumen fluid; TGP, total gas production; tVFA, total VFA concentration; VFA, volatile fatty acid; WSC, water soluble carbohydrate.

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subjected to an analytical procedure. Feed samples used to quantify carbohydrate, N and mineral fractions, gross energy and *in vitro* digestibility, among many assays, are routinely processed in this way. Most international systems for formulating ruminant diets such as the National Research Council (NRC), Institut National de la Recherche Agronomique (INRA), and the Nordic AAT-PVB protein evaluation systems rely on feed compositional data determined using thermally dried samples. Typically, such samples are dried at a temperature between 40 and 70 °C.

Drying methods involving heat can change the chemical composition of a feed. These changes include increases in neutral detergent fibre (NDF Alomar et al., 2003) and lignin contents (Parissi et al., 2005), and decreases in total non-structural carbohydrates (Acosta and Kothmann, 1978). Heat damage due to high temperature drying can cause condensation of sugar residues with amino acids, resulting in formation of indigestible Maillard products, which contribute to the lignin(sa) fraction and are insoluble in acid detergent (Van Soest, 1982). Thermal drying can also result in organic matter losses due to continued activity by plant enzymes (*e.g.*, respiration and proteolysis) and microbial activity (Smith, 1973; Lowman et al., 2002). Hence, the potential reduction in digestible substrate with associated changes in composition of the digestible fraction of herbage could alter the extent of *in vitro* rumen fermentation and the proportions of individual volatile fatty acids (VFA) produced. This, in turn, could alter *in vitro* rumen CH_4 output.

Freeze drying is often considered to be an optimal system for drying grass samples (Deinum and Maassen, 1994; Pelletier et al., 2010). Smith (1973) reported that freeze drying produced non-structural carbohydrate concentrations closest to fresh herbage, while Cone et al. (1998) observed that freeze drying enhanced gas production of substrates compared with thermal drying at 30 or 70 °C during the first 7 h of incubation.

The objectives were to compare effects of freeze drying *versus* thermal drying at 40 °C on the chemical composition and cumulative gas and CH_4 output of perennial ryegrass harvested at sequential stages of the primary growth using an *in vitro* rumen gas production technique.

2. Materials and methods

2.1. Herbage treatments

Perennial ryegrass (*Lolium perenne L.*, Gandalf) was grown at Grange, Dunsany, Co. Meath, Ireland (53°30′N, 6°40′W, 83 m above sea level). Nitrogen fertiliser was applied in mid March at 125 kg/ha. Samples were collected from individual plots ($n = 5$) within replicated blocks ($n = 3$) at five stages of the primary growth (*i.e.*, 12 May, 26 May, 9 June, 23 June, 7 July, 2009—defined as Cuts 1–5, respectively).

2.2. Herbage preparation

After sampling, each sample was stored at −18 °C. When needed, individual samples were thawed at 4 °C for 24 h, bowl-chopped (Muller MKT 204 Special Food Processor, Saarbrücken, Germany) and mixed. Subsamples of each herbage, 200 g, were either freeze dried at −55 °C for 72 h (Scanvac Coolsafe, model no. 55-4, not a programmable tray freeze dryer) or thermally dried in a ventilated oven with forced air circulation at 40 °C for 48 h (preheated to 40 °C before samples were inserted) prior to milling (Retsch Mühle, 5657 Haan, West Germany, GmbH, type-smi, Nr-72267) through a sieve with 1 mm apertures. Thermal drying at this temperature is the standard procedure for forage samples at Teagasc Grange and has been used in other *in vitro* rumen CH_4 studies (Lovett et al., 2006; García-González et al., 2008; Longo et al., 2006).

2.3. Herbage composition

Dry matter (DM) concentration was estimated following drying in a ventilated oven with forced air circulation at 98 °C for 16 h. Determination of *in vitro* organic matter digestibility (OMD) used the Tilley and Terry (1963) technique where the final residue was isolated by filtration (Whatman GF/A 55 mm, pore size 1.6 μm, Whatman International, Maidstone, UK) rather than centrifugation. Both aNDFom (NDF assayed with a heat stable amylase and expressed exclusive of residual ash) and ADFom (acid detergent fibre expressed exclusive of residual ash) were analysed using the filter bag technique (Ankom, 2006a,b, respectively), with ash content determined by complete combustion in a muffle furnace at 550 °C for 5 h. The crude protein (CP) concentration was determined using a Leco FP 528N analyser based on the Protein (crude) in Animal Feeds combustion method (1st action; #990-03) from the Journal of the Association of Analytical Chemists (AOAC, 1990). Acid detergent insoluble N (ADIN) was measured by N analysis on the residue from ADFom assay. Concentrations of water soluble carbohydrate (WSC) was determined using the anthrone method (Thomas, 1977).

2.4. Rumen fluid sampling

Four rumen fistulated steers (682 kg mean liveweight) were individually fed a restricted allowance (0.9 of *ad libitum* intake) of a 600:400 grass silage to concentrate (DM basis) diet, with fresh feed being fed at 10:30 h daily. The composition of the concentrate was 830 g barley, 100 g soyabean meal, 50 g molasses and 20 g mineral plus vitamin (premix)/kg fresh weight. Animals had continual access to fresh drinking water. Rumen fluid (RF) was collected (0.25 L/animal) 1 h