Comparing drying protocols for perennial ryegrass samples in preparation for chemical analysis

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Abstract. Diet formulation for animals requires accurate estimation of feed nutritive value. In order to determine the nutritive value of grass, the moisture in the samples must be removed, with minimum damage to cell structure, and then the dried samples milled, prior to chemical analysis. Generally samples are oven dried. The aim of this study was to verify if differing drying protocols gave diverging results when drying grass samples. The drying protocols investigated were 40°C for 48 hours, 60°C for 48 hours and 95°C for 15 hours in forced convection ovens. Four perennial ryegrass samples were cut to 4 cm from ground level on three occasions in 2012. On each occasion the four grass samples were mixed together thoroughly and divided into four replicates. Each replicate was divided into three sub-samples to give one 100 g-sample per drying treatment. At regular intervals the samples were removed from the oven and the weight recorded. The data were analysed using a mixed model repeated measures procedure in SAS. Time (hours) was used as the repeated measure. A separate dataset of 12 grass samples were dried using both the 40°C and 60°C protocols and then chemically analysed. This dataset was analysed using PROC GLM in SAS. Samples were assumed dry when there was no significant difference in weight between times. All drying protocols gave a similar final dry matter of approximately 156 g/kg. All drying protocols did dry the grass samples adequately as samples dried at 40°C and 60°C were not significantly lighter after 24 hours and samples dried at 95°C were not significantly lighter after 15 hours. There were no differences in ash or crude protein concentration of the samples dried using the 40°C and 60°C protocols. There were differences in the organic matter digestibility, neutral detergent fibre and acid detergent fibre concentrations between the grass samples dried using the different drying protocols

Keywords: Dry matter, drying temperature, chemical analysis, oven, *Lolium perenne*.

Introduction

Accurate determination of the nutritive value of grass is essential for accurate and precise diet formulation for grass-fed animals. The nutritive value of grass is determined by drying and milling the grass samples before chemical analysis. Oven drying in a force draught oven (Faithfull 2003) is generally used for DM determination as large numbers of samples can be processed quickly, cheaply and with less labour compared to freeze drying (Smith 1973). The aim of drying samples prior to chemical analysis is to remove moisture with minimum losses of dry matter (DM) and chemical constituents (Smith 1973; Johnson 1978). Drying at a temperature that is too high can cause modification or destruction to some plant constituents (Johnson 1978). Similarly drying at a temperature which is too low may cause DM losses due to respiration and enzymatic conversions (Smith 1973). There is often a compromise between temperature choice and length of drying time (Faithfull 2003).

Grass samples are generally dried at a temperature of between 40 and 80°C for different lengths of time (Smith 1973; Driehuis *et al.* 2001; Faithfull 2003; Purcell *et al.* 2011). There are different drying protocols operating in different countries. For example Teagasc, in Ireland, oven dry grass samples at 40°C for 48 hours prior to chemical analysis (McEvoy *et al.* 2010; McCarthy *et al.* 2012). If only the DM is required then grass samples are oven dried at 95°C for 15 hours. In contrast, INRA in France oven dry grass samples at 60°C for 48 to 72 hours (Andueza *et al.* 2011).

The aim of this study was to determine if different drying protocols gave different results when drying grass samples in preparation for subsequent chemical analysis.

Methods

The study took place at Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (50° 09'N; 8°16'W). The soil type was a free draining acid brown earth of sandy loam-to-loam texture. On three different occasions (17th January, 7th February and 21st February) perennial ryegrass (*Lolium perenne* L.) samples were collected from one paddock with an average pre-grazing herbage mass > 4 cm of 1 t DM/ha. The samples were cut to 4 cm from ground level at random across the paddock using a Gardena hand shear (Accu 60,

Gardena International GmbH, Ulm, Germany). On each collection date the samples were mixed thoroughly and divided into four replicates. Each replicate was then divided into three sub-samples. This meant there were four sub-samples per drying protocol per collection date. The three drying protocols were oven drying at 40°C for 48 hours, 60°C for 48 hours and 95°C for 15 hours in forced convection ovens. Two Carbolite ovens (Carbolite Limited, Hope Valley, S33 6RB, UK) were used to dry the samples at 40°C and 60°C. A Binder FED 720 oven (BINDER Inc. (North America) Bohemia, NY 117 16, USA) was used for the 95°C protocol.

The porous foil containers used to hold the grass samples were placed in the 95°C oven the night before grass sample collection to ensure that the trays were completely dry for use the following day. Approximately 100 g of grass were weighed onto each tray and the weight recorded. The ovens were pre-heated for 1 hour prior to inserting the foil trays containing the grass samples. Each sample was placed on a separate shelf in the middle of the oven. The times for the drying protocols were extended beyond the time specified in the protocol, to determine if any change in DM occurred after 48 hours for drying at 40 and 60°C and after 15 hours for the 95°C protocol. The 95°C protocol was extended to 24 hours, the 60°C protocol was extended to 75 hours and the 40°C protocol extended to 82 hours. Sample weighing started at 0 hours, finished at 82 hours and after each weighing the trays were placed back in the oven. At 0, 3, 7, 10, 13, 15, 24 hours all samples were removed from the oven and the weight recorded. At 27, 31, 34, 48, 51, 55, 58, 72, 75 hours the samples drying at 40 and 60°C were removed from the oven and the weight recorded. At 79 and 82 hours the samples drying at 40°C were removed from the oven and the weight recorded. A separate dataset of 12 samples were dried using the both the 40 and 60°C protocols. These samples were then analysed for ash (AOAC, 1995; method 942.05), crude protein (CP; Leco FP-428; Leco Corporation, St. Joseph, MI, USA), neutral detergent fibre (NDF) and acid detergent fibre (ADF; AOAC 1995; method 973.18; using sodium sulphate for the NDF; ANKOM™ technology, Macedon, NY,USA). The samples were analysed for OMD using the method of Dowman and Collins (1982) as modified by Morgan *et al.* (1989). Organic matter digestibility was determined in triplicate in all samples.

Statistical analysis

Data were analysed for normality using PROC UNIVARIATE in SAS and were found to be normally distributed. Data were analysed using the mixed model repeated measures procedure (PROC MIXED) in SAS (2002). Day, temperature, time and the interaction between temperature and time were all included in the model. Time was included as the repeated measure and sub-sample within temperature as the random variable. For all data the random statement specified the compound symmetry structure. The chemical composition of the 12 samples dried using the 40 and 60°C protocols were statistically analysed using PROC GLM in SAS (2002) with drying temperature included as a fixed effect. The Tukey-Kramer multiple range test was used for mean separation (P<0.05).

Results and discussion

After 24 hours the samples drying at 95°C were removed from the oven, after 75 hours the samples drying at 60°C were removed from the oven and after 82 hours the samples drying at 40°C were removed from the oven (Fig. 1). The final DM% was similar for all three drying protocols (P>0.05). Drying at 95°C for 15 hours gave a final DM of 153.8 g/kg (± SEM 6.8), drying at 60°C for 48 hours gave a final DM of 155.2 g/kg (±7.0) and drying at 40°C for 48 hours gave a final DM of 159.4 g/kg (± 8.0) (average for the three collection dates). Therefore, all three protocols were acceptable for calculating DM%. Drying at 95°C for 15 hours is only used in Ireland for determining DM content as the material dried at this temperature may be unsuitable for chemical analysis.

Drying at temperatures above 80°C can cause thermo-chemical degradation according to a review of literature by Smith (1973). This agrees with Johnson (1978) who said that elevated temperatures can destroy or modify some plant constituents such as amino acids. Faithfull (2003) said that when determining only the DM%, the drying

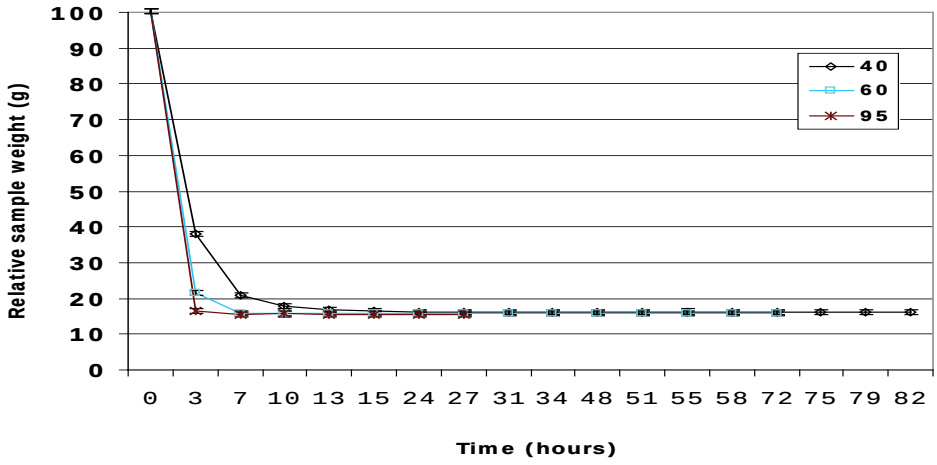

Figure 1. The average dry matter (DM) % (±SEM) of four perennial ryegrass samples collected in January and February 2012, when dried at three temperatures (40, 60 and 95°C) for up to 82 hours.

Table 1. Chemical composition of grass samples dried at 40 °C for 48 hours and 60 °C for 48 hours.

	40 °C	60°C	SEM	*P*-value
Ash (g/kg)	91.4	85.0	4.50	ns
Crude Protein (g/kg)	220.5	215.9	3.29	ns
OMD (g/kg)	752.6	820.4	4.79	***
NDF (g/kg)	424.6	451.4	18.98	**
ADF (g/kg)	239.2	229.5	6.91	*

temperatures and times are higher and shorter than when drying prior to chemical analysis. After reviewing the literature, Smith (1973) concluded that drying below 50°C prior to chemical analysis is undesirable, which the author postulated was due to DM losses incurred by respiration and enzymatic conversions. Contrary to that review, the results of the present study found that drying at 40°C for 48 hours is acceptable for grass samples prior to conducting certain chemical analyses as it gave a similar final DM%, CP and ash concentration to drying at 60°C (Table 1). Similarly, Purcell *et al.* (2011) found that there was no difference in water soluble carbohydrate, CP or ash content or in the OMD of grass which was dried at 40°C and freeze dried. Freeze drying is considered one of the best methods for drying material as it removes water with minimum damage to cell structure (Faithfull 2003). However, in this study the OMD, NDF and ADF concentrations differed between the grass samples dried at 40°C and 60°C (Table 1). The difference in OMD is likely due to the loss of WSC caused by respiration which Deinum and Maassen (1994) found in samples dried at 30°C. The lower OMD of the samples dried at 40°C indicates that 40 °C may be too low a temperature to dry at, agreeing with the review of Smith (1973). Purcell *et al.* (2011) found that NDF and ADF was lower in the samples that were dried at 40°C compared to freeze drying, which was the opposite to what Alomar *et al.* (2003) and Parissi *et al.* (2005) found. Our results found that NDF was lower and ADF higher in samples dried at 40°C compared to 60°C, indicating that there may have been a loss of hemicellulose thus reducing NDF when samples were dried at 40°C. This loss in hemicellulose may also have contributed to the lower OMD of the samples dried at 40°C. Further work should be conducted to determine which method is more accurate.

The rate of drying decreased in the order of 95, 60 and 40°C. Samples were assumed dry when there was no significant difference in weight between times. Grass samples dried at 40 and 60°C were not significantly lighter after 24 hours and samples dried at 95°C were not significantly lighter after 15 hours (P>0.05, Fig. 1). This demonstrates that all drying protocols dried the samples adequately. Similarly grass silages were found to be dry after 24 hours at 100, 70 and 40°C as a constant sample weight had been achieved (Minson and Lancaster 1963). In contrast, Deinum and Maassen (1994) concluded that drying at 30 or 50°C was not acceptable due to respiration losses and long drying times of 48 hours for stems of fodder radish, lucerne, Italian ryegrass and maize silage. The quick drying times found in the present study suggest that 40 and 60°C are acceptable temperatures for drying grass samples prior to chemical analysis. Drying may be enhanced by having the samples in a thin layer and in a porous container allowing the sample to be exposed as much as possible (Smith 1973; Pelletier *et al.* 2010). The samples in the present study were held in porous containers and well spread out which may have resulted in quick drying of the samples. Differences in the chemical composition and physical structure of the samples may result in differences in drying time. Deinum and Maassen (1994) used maize silage, fodder radish, Italian ryegrass and lucerne compared to perennial ryegrass used in the present study. These differences in materials used combined with differences in sample size may explain the differing results. Deinum and Maassen (1994) used a sample size of 500 g compared to 100 g in the present study. A larger sample size could result in the sample taking longer to dry as it would take the air longer to circulate around. Good sampling technique is essential to minimise error in an experiment (Jones and Moseley 1993). They recommend thoroughly mixing the sample, dividing into quarters and randomly collecting a sub-sample of 300-500 g. The present study used four 100 g samples per treatment.

Conclusion

The results of the present study indicate that the three drying protocols (drying at 40°C for 48 hours, 60°C for 48 hours and 95°C for 15 hours) adequately dried grass samples to a similar final weight. Drying at 95°C is likely to be only suitable for DM determination, as previous studies indicated that the material drying at 95°C may be unsuitable for chemical composition analysis. Differences in OMD, NDF and ADF concentrations in grass samples dried using the 40°C and 60°C protocols suggests that future work should investigate which method is more accurate.

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