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Sample preparation of *Medicago sativa* L. hay for chemical analysis

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Abstract

The objective of this study was to quantify the effect of the grinding procedure on the moisture and crude protein concentration of a ground *Medicago sativa* L. hay sample for quality grading. An additional aim was to investigate the accuracy of electronic moisture testers (EMT). Variance of analyses revealed significant differences in moisture concentration between ground (CV = 16.1%) and unground (CV = 27.4%) samples ranging from 14.7 up to 41.1% of the unground sample. The grinding process had a non-significant influence on the CP concentration of the final grounded product. EMT failed to accurately predict moisture concentration around the moisture area of critical concern (16% and higher) where heat and/or mould damage are likely to occur. It was concluded that analytical moisture standards for *Medicago sativa* L. hay should be based on the original moisture concentration of samples in the unground state to be relevant for quality grading.

Keywords: Lucerne hay, sampling, sample preparation, moisture concentration, crude protein
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Introduction

Sampling procedure for lucerne hay is well defined in the literature (Bath & Marble, 1989; Martin *et al*., 1992; Putnam, 1998; Sheaffer *et al*., 2000). However, very little research has been done on sample preparation for lucerne hay quality grading. According to Williams & Norris (2001), sample preparation is defined as the transformation of the sample into the form in which it will be analysed, without causing any changes in functionality or composition other than in moisture content. This process often calls for some type of size reduction.

Knowledge of lucerne hay moisture is, however, critical for proper harvesting quality assessment and storage. Several subjective, physical, analytical (chemical and near infrared reflectance spectroscopy) and industrial methods for determining the moisture content of lucerne hay exist.

Electronic probe type moisture testers (EMT) operate on the principle of electrical resistance, utilising the relationship between the moisture content of the material and its conductivity (Shewmaker & Thaemert, 2004). These probe-type meters are, however, subject to error due to various external factors. These factors include variation in bale density, the type of forage, whether it is plant moisture or dew moisture and ambient temperature (Shewmaker & Thaemert, 2004).

Alternatively, chemical or near infrared reflectance spectroscopy (NIRS) analysis of moisture includes the transformation and reduction of the sample size by means of a laboratory grinder. Grinding could, however, be a major cause of variation in moisture and other analytical results (Groenewald & Köster, 2005). Furthermore, moisture loss during the grinding process is especially important for NIR scanning due to the fact that water is a strong absorber of NIR light. The degree of hydration may influence the optimum area of the NIR spectrum where the absorbers of specific constituents occur, thus affecting the whole NIR wavelength region (De Boever *et al*., 1996; Williams & Norris, 2001). Therefore, it could affect the predicted results of all the other parameters (CP, ADF, NDF, etc.).

The grinding procedure could not only alter the moisture status of the sample, but also affect changes in composition such as crude protein (CP) due to losses in the form of dust. According to Williams & Norris (2001) dust generated in the grinding process and the incomplete-ground residue in the grinder chamber is probably the largest cause of contamination between samples, especially in routine operations when a large number of samples are processed. Groenewald & Köster (2005) are of opinion that the product lost is not of

the same composition as the product before grinding. Therefore, the final sample to be analysed might not be really representative of the baled lucerne hay that will be fed to animals.

In the available literature no guidelines could be found for moisture and dry matter (DM) losses of lucerne hay during sample preparation. Most grinding statistics are based on grains (Williams & Norris, 2001) which lead to several assumptions regarding forages in general.

The objective of this study was to quantify the effect of the grinding procedure on the moisture and crude protein content of the ground *Medicago sativa* L. hay sample for quality grading. An additional aim was to investigate the accuracy of electronic moisture testers.

Materials and Methods

Forty-six samples of lucerne hay (*Medicago sativa* L.) (n = 46) were obtained from several commercial irrigation farms at different locations in the Douglas and Hartswater area of South Africa (September 2006 to May 2007). The samples represented lots that were selected at different stages of maturity. A moisture range as broad as possible was obtained by means of a FARMEX electronic bale moisture probe (FARMEX, 1205 Danner Drive, Aurora, Ohio 44202). These samples were also obtained from different bale types namely small and large rectangular bales, as well as round bales. Each lucerne hay sample was a composite of 20 core samples (± 12 g dry weight) from the same cutting (lot), with a LTC (Lucernetech Consult CC., Hopetown, South Africa) forage sampler. Accordingly, the moisture reading of each sample was the average value of these 20 bales used to collect the core samples. In an effort to minimise moisture loss the unground samples were immediately sealed in airtight containers and stored in a refrigerator below 5 °C for subsequent grinding and chemical analysis.

The 46 samples stored in a refrigerator below 5 °C were exposed to room temperature (25 °C) and a relative humidity (RH) of 56% for one hour to reach temperature equilibrium prior to grinding. Due to the nature of lucerne hay, care had to be taken to protect the natural ratio of leaves and stems. Every attempt was made in this study to obtain a representative sub-sample from each hay sample collected to obtain a representative moisture value for lucerne hay in the unground form. Each sample was thoroughly mixed and a representative sub-sample obtained (n = 46) for subsequent moisture analysis. The remainder of each sample was ground through a 1-mm screen using a LM 3100 laboratory grinder (Perten Instruments AB, Huddinge, Sweden) most widely used in South Africa. .

Separate samples (n = 5) were used to pre-warm the grinder to a constant temperature of 46 °C before grinding the experimental samples (n = 46). The samples were milled for a total of ± 100 seconds with a waiting period in between samples, lasting ± 120 seconds each, to minimise temperature fluctuations within the sample and grinder. Temperature readings in the sample and grinding chamber were taken directly after grinding each sample, by means of a mercury thermometer. The material left in the grinding chamber after grinding each sample was quantitatively collected and stored in a refrigerator below 5 °C for subsequent CP analysis. Additionally, the dust left in the dust bag originating from each sample was collected by thorough cleaning of the dust bag. The weight of the dust was determined by weighing the bag before and after grinding.

Moisture in the ground and unground samples (n = 46) was determined by forced-air oven drying at 98 °C for 24 h (AOAC, 2000). Crude protein concentration (CP: N x 6.25) of the residue in the dust bag and material remaining in the grinder were determined by means of the procedures described by AOAC (2000) using a Leco FP-528 Nitrogen Combustion Analyser (Leco. 3000 Lakeview Avenue, St. Joseph, MI 49085).

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (2002-2003). Descriptive statistics namely the mean, standard deviation, coefficient of variation, minimum and maximum values were calculated for the quantitative variables (SAS, 2003).

Results and Discussion

The results when using an EMT to determine the moisture content in lucerne hay are set out in Table 1. An over-estimation of the moisture content of lucerne hay occurred at especially the maximum moisture level. These results were also reflected by the coefficient of variation values. EMT measures the resistance or conductivity in the hay. A small increase in bale density and/or moisture on the surface of the hay may increase conductivity dramatically (Shewmaker & Thaemert, 2004). This increase, due to surface moisture and bale density, results in an over-estimation of the moisture in lucerne hay. The average over-prediction of the moisture content in baled lucerne hay by the EMT compared to unground samples, in Table

1 was in agreement with the ± 5% (% moisture units) reported by Shewmaker & Thaemert (2004). However, the average moisture content of the same samples after grinding was under-predicted by 8.5% (Table 1), when estimated by the EMT.

Table 1 The effect of an electronic moisture tester and grinding on the moisture content of *Medicago sativa* L. hay

	Unground sample*	Ground sample*	Farmex [a]
Minimum (%)	6.8	5.8	8
Maximum (%)	18	10.6	37
Mean (%)	11.5	8.5	17
Standard Deviation	3.1	1.3	7.3
Coefficient of variation	27.4	16.1	41.8

* Normal distributed (P >0.05).
[a] Electronic moisture tester (FARMEX, 1205 Danner Drive, Aurora, Ohio 44202).

Even though the coefficient of determination between analytical measured moisture results on unground samples and values predicted by EMT was significantly high (r^2 = 0.79, P <0.0001), the EMT failed to accurately predict moisture content around the critical moisture levels of 16% and higher. Bath & Marble (1989) stated that less than 16% moisture will prevent heat damage and moulding.

From the results in Table 1 it is evident that the grinding of lucerne hay resulted in moisture losses. Analysis of variance revealed significant (P <0.0001) differences in the moisture concentration between the two treatments, which was also confirmed by Tukey's multiple tests. The mean sample moisture loss for the current study (27.5% of original moisture content) was high and somewhat unexpected. These losses were more pronounced for lucerne hay with high moisture content. In accordance with these results, Williams (2007) stated that moisture loss during the grinding process often causes underprediction of moisture content of the product sampled for analysis. According to Williams & Norris (2001) additional factors could influence the losses in moisture content namely the type of grinder, screen size, changes of revolutions per minute (RPM) of grinders, grinder maintenance and number of samples ground at the same time. According to the Shapiro-Wilk test for normality, the moisture content of lucerne hay samples was normally distributed (P >0.05) across the range for both treatments (ground and unground). The results in Table 1 also show that grinding lucerne hay samples results in a lower CV for moisture content. Such lower variation was expected because heating generated by the grinding process of samples generally stabilises the moisture content of fibrous material (Groenewald, C.A., 2007, Pers. Comm., Divisional Director, Scinetic, Centre of scientific technology, a division of Afgri operations. 252 Jean ave., Centurion, South Africa).

The incomplete ground lucerne hay residues remaining in the grinder varied from 0.36 to 3.16% DM. The average loss was slightly lower than the 2% DM loss reported by Williams (2007) for grain, using a similar grinder.

Sample lost due to dust generated during the grinding process was non-significant (P >0.05) negatively correlated (r = -0.1) with moisture concentration of the unground sample. This non-significant (P >0.05) decrease in dust generated during the grinding of higher moisture samples was, however, unexpected due to a customary decrease in dust generation with an higher moisture content. The high observed CV observed for dust could have contributed to these results.

The variance of analysis revealed non-significant (P = 0.6452, CV = 8.0094) differences in the CP concentration among the ground and unground samples, and is supported by Tukey's studendised range test. Surprisingly, CP concentration (% DM) of dust (14.78%) generated in the grinding process was significantly (P <0.0001) lower than that of the original unground sample (19.78%). This could probably be explained by the gusting of the lower specific gravity and CP-containing fibre particles into the dust bag during the grinding process.

It is clear that because of the relatively small loss in dust (1.07%) and its lower CP concentration (14.78%), very little difference was observed in the CP concentration of lucerne hay samples with or without

undergoing the grinding process. A virtually perfect positive relationship (r^2 = 0.99; P <0.0001) was observed between the CP concentration of unground and ground samples. Thus, based on these results, it is evident that dust generated in the grinding process had a non-significant (P >0.05) influence on the CP concentration of the end product.

Conclusions

From the results of the present study and those in the literature with lucerne hay, it seems that moisture losses during grinding are more prominent for lucerne hay (27.5% of original moisture concentration) with a high moisture concentration. Based on these results it is evident that analytical moisture standards for lucerne hay quality grading should be based on the original moisture concentration of samples in the unground state. The CP concentration of lucerne hay was, however, not influenced by grinding. However, the effect of grinding on other chemical parameters warrants further investigation.

Results from the present study also point out that moisture values predicted by electronic moisture testers fail to accurately predict moisture concentration around the critical moisture level of 16% and higher.

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