Development of a laboratory procedure to determine dry matter in maize forage

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Abstract

Although laboratories have performed dry matter (DM) assessments for several years on maize forage samples, there has not been an appropriate "standard method" to follow. Each laboratory has adapted existing methods in order to provide this specific service. Concerns about the methodology and accuracy of this testing service arose in 2001, resulting in the formation of the Forage Trading and Development Group, who undertook to prepare a suitable Code of Practice. Although determination of DM in the laboratory is a relatively simple process and the major source of error lies with the original sample collection (at the source), there are laboratory issues that could also contribute to accuracy. Factors that could affect the accuracy and reproducibility include: the amount of sub-sample taken for analysis, the sub-sampling method used, the drying temperature and the length of drying time. Initially, 12 New Zealand (NZ) laboratories known to be providing this service were contacted, from whom basic information was requested as to how they currently performed a maize forage DM test. A consensus, draft method was prepared and distributed to all laboratories. This method, now proposed as the method to be used by NZ laboratories, requires a minimum of 500g of fresh sample, dried at 105 °C until a constant dry weight is attained. Within-laboratory sub-sampling by riffle-box was found to be superior to the manual quartering technique. In addition, inter-laboratory comparison programmes were conducted during both the 2001-02 and 2002-03 maize silage seasons. Repeatability (within laboratory variation) and reproducibility (inter laboratory variation) were similar, with standard deviations of 0.38 % and 0.45 %, respectively, in 2002, and 0.25 % and 0.34 %, in 2003.

Additional key words: silage, fair-trading, hand quartering, riffle-box, stack, oven drying.

Introduction

There has been a marked increase in the trading of maize forage in New Zealand in recent years. Maize forage is normally sold on the basis of dry matter (DM), which involves recording the wet weight of the harvested crop, collecting a representative sub-sample from the bulk stack or pit prior to ensiling, and then a laboratory determination of the percentage DM in the fresh sample. The total DM is then readily calculated by multiplying the fresh weight by the DM factor.

There is concern of some involved in the industry about the accuracy of the DM determination sub-sampling of this process, as small deviations from the "true" value can have a significant effect on the value of the crop (DeFilippi, 2004). In 2001 an AgMARDT grant was obtained to set up a Forage Trading and Development Group (FTDG). This group's primary task was to research and develop methodologies for the accurate sampling and testing of maize forage. Issues relating to field sampling have been presented elsewhere (DeFilippi, 2004), and this paper describes the development of an appropriate laboratory method for the DM determination.

Determining the DM content of feeds is generally regarded as a reasonably

straightforward test, and several publications provide standard methods for this analysis (AOAC International, 2000; and National Forage Testing Association, 1997). The most common approach to determining dry matter is from weight loss by oven drying a sample. This method assumes that only water is being volatilized at the temperatures used and has the advantage of being relatively easy to perform. There are alternative procedures, for example, microwave oven drying (National Forage Testing Association, 1997) and chemical extraction of the water (AOAC International, 2000). These methods were not considered practical or appropriate, because of the nature of freshly harvested maize forage, and the large volumes of samples being received at testing laboratories during the harvest season. Near infra-red spectroscopy (NIRS) is also used to determine dry matter (AOAC International, 2000; and National Forage Testing Association, 1997), but this still requires a reference wet chemistry procedure to calibrate the NIRS instrument. Published methods using NIRS involve small, ground samples with typically 6 – 16 % moisture, and are not suitable for high accuracy analyses of fresh maize forage samples. The oven-drying method was identified as the best approach to use: because of the relative simplicity of the technique, it lends itself to processing of large volumes, and it is the method currently in widespread use in New Zealand.

The heterogeneity of maize forage (cob and stover) creates challenges for the analyst. Many of the published methods are for materials that have been ground prior to analysis, and sample weights of 2 g are commonly recommended. The NFTA Procedures Manual (National Forage Testing Association, 1997) method utilizing microwave drying states that 100 – 200 g of sample is to be used for the analysis. For many forages, this quantity may be sufficient, but for a heterogeneous sample like maize fodder, where highly precise results are needed for trading purposes, this may not be sufficient. Pioneer Technologies in the United States process 700 g of chopped maize forage when evaluating maize varieties (pers. com. D. Sapienza, Pioneer International).

A suitable laboratory procedure was developed and distributed amongst the laboratories, and an Inter-Laboratory Comparison Programme (ILCP) was instigated. This involved carefully dividing a bulk sample into a number of smaller, equivalent samples, and a sub-sample being sent to each participating laboratory. The DM results were reported back, and collated. This exercise allowed each laboratory to see how it performed compared to the other laboratories, and also showed how NZ laboratories are performing overall (individual laboratory's confidentiality was respected, and only collated results were disclosed to the participating laboratories.)

Methods

In August 2001, all twelve laboratories known to have performed maize forage DM analyses were contacted and accepted an invitation to become involved in a project to develop a standard analytical procedure. The first step was to find out what methods laboratories were currently using, and to see if a "consensus" method could be derived.

A questionnaire was sent out, to determine what was currently being done, and from the responses, a draft standard method was compiled. A drying temperature of 105 °C was adopted, as most laboratories already used this temperature and it is a standard temperature used for the determination of DM (National Forage Testing Association, 1997). The two variables that were suspected to be critical, were the quantity of sample analysed and the drying time.

Drying conditions experiment

The following experiment was conducted at Hill Laboratories to check these variables:

a) A sample of freshly harvested maize forage (35 kg) was sub-sampled on a clean vinyl surface by a quartering technique, to yield 32 sub-samples of approx. 1 kg. (1 kg was thought to be the likely maximum sample size to be routinely processed).
b) Three of the 1 kg bags were recombined, mixed, then further sub-sampled to yield 6 x 500g sub-samples.
c) One of the 500 g portions and a further 1 kg sub-sample were recombined and mixed, then sub-sampled to yield 6 x 250g sub-samples.
d) Fifteen subsamples (5 x 1 kg, 5 x 500g, 5 x 250g) were placed in a pre-weighed, aluminium foil trays, re-weighed, then transferred to a forced air convection oven. The 1kg and 500g sub-samples were in 300 x 230 x 60 mm trays, and the 250g sub-samples were in 200 x 140 x 45 mm trays.
e) After 16, 24, 40, 48 and 72 hours, the trays were removed from the drier, allowed to cool, and then immediately weighed. They were then returned to the oven drier to continue the experiment.

Smaller sized trays were used for the 250 g sub-samples, in order to minimize space required in the driers, which was limited at that time of the year.

The results for the 500 g and 1 kg sub-samples were slightly surprising, and so in the following season (2002 – 2003), the experiment was repeated, taking ten replicates instead of five.

Inter-laboratory comparison programmes (ILCPs)

Some of the 1 kg sub-samples of the bulk sample were also used for the ILCP. One bag was immediately couriered to each of the twelve participating laboratories. Results were reported back and the mean and standard deviation calculated. Participating laboratories were then sent a summary of all the results, without identifying the source of individual results.

The ILCP was again repeated at the start of the 2002 -2003 season.

Sub-sampling procedure

Samples submitted to the laboratory are often in excess of 1 kg, and need to be sub-sampled. The hand quartering approach was initially used in this investigation. This involves placing the sample in a shallow tray, mixing it well, then dividing it into four quadrants. Two diagonally opposite quadrants are combined to give one sub-sample, and the remaining two quadrants then combined. This method yields two equivalent sub-samples from the initial sample. One sub-sample is used for the testing while the second is retained in case a repeat analysis is necessary.

An alternative method to divide a sample into two equal sub-samples is to use a gated riffle-box. In 2003, a riffle-box capable of processing 1 kg maize forage samples was purchased. The riffle-box comprised a hopper with eight chutes in the base, four directing the sample into one tray, and the other four into a second tray.

In order to establish whether one sub-sampling technique was superior to the other, an experiment was undertaken using 20 randomly selected 1 kg samples; ten of which were divided by hand quartering, and ten

divided using the riffle-box. The DM of each sub-sample was determined by the draft testing protocol, yielding 10 pairs of data for the hand-quartered samples and the riffle-box samples. Using such pairs of data, the standard deviation for both groups of data was estimated (International Accreditation New Zealand, 2003).

Results and Discussion

The questionnaire sent to all laboratories at the start of the investigation revealed that everyone was using an oven drying procedure, but that there were variations in the weights being analysed (100 – 1000 g), temperatures used (95 – 135 °C), and drying times (5 – 72 hours). Information about sub-sampling procedures was not sought at that time. The initial draft method prepared was largely a "consensus" of all these methods, taking into account practical issues faced by testing laboratories.

Drying conditions experiment

The results of the drying time and sample weight experiment, undertaken in March 2002 at Hill Laboratories, is given in Table 1.

Table 1. Summary of results of DM vs Drying Time and Fresh Weights Analysed.

Fresh Weight Analysed		16 hrs drying @105°C	24 hrs drying @105°C	40 hrs drying @105°C	48 hrs drying @105°C	64 hrs drying @105°C	72 hrs drying @105°C
1 kg							
	Mean (%):	38.7	31.9	30.7	30.7	30.6	30.6
	S.D. (%):	4.9	1.6	0.4	0.4	0.4	0.4
500 g							
	Mean (%):	31.1	30.8	30.7	30.6	30.6	30.6
	S.D. (%):	0.4	0.4	0.4	0.4	0.4	0.4
250 g							
	Mean (%):	30.7	30.4	30.4	30.3	30.3	30.3
	S.D. (%):	0.8	0.8	0.8	0.8	0.8	0.8

These results showed that, in order to reach a constant weight, the 1 kg sub-sample took 64 hours, while the 500 g and 250 g sub-sample both took 48 hours. Because of the volumes of samples being submitted during the harvest season, it would be highly desirable for the drying to be completed in 24 hours, i.e. to be able to process the samples submitted each day in a 24 hour period.. For the 1 kg sample, the sample still contained 1.6 % moisture, but the 500 g and 250 g sub-samples contained 0.2 and 0.1 % moisture, respectively, after 24 hours. The latter two could be considered to have virtually attained a constant weight after 24 hours drying at 105 °C.

An obvious difference between the samples of different weight was the thickness of the sample layer in the drying trays. A quick experiment confirmed (as expected) that samples dried much more quickly as a thin layer on a large tray, compared to a thick layer in a smaller tray. But while very large trays would dramatically improve the rate of drying, they would not be practical, as their large "footprint" would dramatically limits a laboratory's daily capacity.

It also became apparent that the drying time not only depends upon the tray dimensions, but also the sample loading and the efficiency of air movement within the oven. These factors

will differ from laboratory to laboratory, and so it was decided that the method should not specify an exact drying time, but state that the sample should be "dried to a constant weight", with the above information to be used as a guide to drying times likely to be required. Each laboratory will need to establish how long the drying time should be for their particular circumstances.

The standard deviations, once the DM was close to the final constant value, showed markedly better precision when increasing the sample weight analysed from 250 g to 500 g (0.8 % c.f. 0.4 %), but increasing it further to 1 kg produced no discernable increase (0.4 % c.f. 0.4 %). No improvement at all seemed a little surprising, and the following season, the experiment was repeated using ten replicates. The results are shown in Table 2 and again showed little improvement in accuracy when increasing the sample weight from 500 g to 1 kg. This experiment confirmed that 500g should be the recommended sample weight to be analysed in the laboratory.

Table 2. Standard deviation of the variation within sample size analysed.

	250 g Sample Analysed	500 g Sample Analysed	1 kg Sample Analysed
2002 Trial (5 Replicates)	0.79	0.38	0.38
2003 Trial (10 Replicates)	0.63	0.25	0.29

Inter-laboratory comparison programmes:

Results were received from all 12 laboratories invited to participate in the first (2002) ILCP. Nine used the new, proposed method, two did not and one was unknown. In the following season, the same 12 laboratories participated in another ILCP. Unfortunately, one laboratory received their sample several days late, so their result was omitted from the final data analysis.

Table 3. Summary of inter-laboratory performance in ILCP Rounds

	2002 Season	2003 Season
All Data:		
Range of results (%)	35.6 – 37.7	32.5 – 34.0
Mean (%)	36.4	33.1
Standard deviation (%)	0.52	0.45
One Outlier removed:		
Range of results (%)	35.6 – 36.9	32.5 – 33.3
Mean (%)	36.3	33.0
Standard deviation (%)	0.45	0.34

In both rounds, there was one result that that appeared to be an outlier, and for interest, these results were removed, to see how it affected the remaining data. An inter-laboratory summary of the DM results over both seasons is presented in Table 3.

The most important result here is the standard deviation, as this shows how good the agreement is between laboratories. The inter laboratory variation was 0.45 % and 0.34 %, for the two years. These results are extremely pleasing, especially when variability studies undertaken by one laboratory with the same samples produced standard deviations of 0.38 and 0.25 %, respectively (refer Table 2). Normally, the within laboratory variation (i.e. the repeatability of the test) is often less than half of the inter laboratory variation (i.e. the reproducibility of the test). That the reproducibility is only slightly higher than the repeatability apparently reflects the robustness and simplicity of the method. Another observation from the 2003 round is that once the outlier was removed, the other 11 results were no more than 0.5 % lower or 0.3 % higher than the mean DM value. The outlier was also only 1.0% above the mean DM.

The slightly better performance observed in the 2003 round compared with the 2002 round may have been due to the participants becoming more proficient with the proposed method. However, it is more likely due to differences in the homogeneity of the samples selected for the rounds. A similar "improvement" was also observed in the repeatability studies undertaken by one laboratory, using the same bulked samples (see Table 2).

Sub-sampling procedure

The two sub-sampling procedures provided standard deviations of 0.21 % (riffle-box) and 0.38 % (hand quartered), respectively. The lower SD from the riffle-box sub-samples compared with the hand quartered sub-samples was a little surprising, as the hand quartering technique did appear to evenly divide these samples. Based on these results, it is our recommendation that, if possible, a riffle-box should be used for sub-sampling in the laboratory. But if such a device is not available, then hand quartering may be used.

Also of concern is the time delay between harvesting the sample and beginning the analysis. Samples should be kept in an airtight bag, to prevent loss of moisture from the sample. They must also be kept cool, to retard the ensilation process, where carbohydrates are converted to volatile products. Ideally, samples should be delivered to the laboratory within a few hours of sampling. If this is not possible, they should be either refrigerated or frozen.

Conclusions

A method has been developed for the laboratory determination of the DM content of maize forage samples for trading purposes. Important variables are the drying temperature (105 °C), the amount of sample analysed (500 g) and the drying time (dry to constant weight). Sub-sampling should be done using a riffle-box; but if unavailable, hand-quartering is acceptable. The full laboratory method has been given in a draft Code of Practice (Foundation for Arable Research, 2002), and is also available for downloading from the Foundation of Arable Research's website at www.far.org.nz.

Developing the method had to also consider practical constraints, such as the drying capacity of laboratories in NZ. This meant the method must use the smallest amount of sub-sample possible, while maintaining good analytical precision and accuracy, and then drying it for the shortest possible time (so driers can be emptied and re-used for the next set of samples). While extra drying time or larger samples will undoubtedly provide a greater "safety margin" in the method, it will

be at the expense of test capacity. In addition, it is important that the original samples received by the testing laboratories are at least 1 kg in size. This allows for accurate sub-sampling and provides a 'spare' sample which can be used if re-testing is required.

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