applied (or as a % of the applied N), depending on the land-application method used.

A slower rate of fertiliser N release generally increases N use efficiency and pasture yields (Zaman *et al.* 2009). Organic N is a slow-release form of N, so a higher organic N content can be expected to increase long-term pasture production.

Reductions in nitrogen fertiliser application rates may therefore reduce N loss from land-applied dairy shed effluent, and may increase pasture production on paddocks receiving effluent.

Further research is needed to confirm these effects (either more broadly over a larger number of farms or in a controlled study), to better understand the mechanisms driving these observations, and to investigate the wider farm system implications of these findings.

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Post-sampling procedure affects the measured nutritive value of perennial ryegrass (*Lolium perenne*)

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Abstract

Estimates of pasture nutritive value are affected by how samples are stored and dried before laboratory analyses. To determine which post-sampling procedures best preserved nutritive value, a range of procedures were compared for one diploid and one tetraploid perennial ryegrass cultivar in June 2016 and April 2017. Treatments included different combinations of transportation from the field to the laboratory either in liquid nitrogen or in a chilly-bin with ice-packs, storage at room temperature (19°C), a chiller (3°C), standard freezer (-18°C) or a -80°C freezer, and drying either in a freeze-drier or an oven at 65°C for 48 hours. Samples were sent to a commercial laboratory for near-infrared reflectance spectroscopy analysis.

Values were higher for one or more of metabolisable energy, soluble sugars, non-structural carbohydrate, and organic matter digestibility, and lower for neutral detergent fibre and ash, when comparing transportation in liquid nitrogen with a chilly-bin, freezer storage (-80°C and standard freezer) with chiller and room temperature storage, and freeze-drying with oven-drying ($P<0.01$). Freeze-drying was the most critical factor in preserving the nutritive value.

Keywords: freeze-drying, oven-drying, liquid nitrogen, pasture sample storage, nutritive value preservation

Introduction

Perennial ryegrass (*Lolium perenne*) is the single most important plant species to the New Zealand economy, generating more than $14 billion/annum (http://mpi.govt.nz/document-vault/14527). The nutritive value of perennial ryegrass-based pastures is one of the key drivers of livestock performance (Feedsmart 2012); accurate information on pasture nutritive value is critical to predict livestock performance and farm profitability.

Assessment of nutritive value for perennial ryegrass-based pastures requires the harvested pasture sample to be transported from the paddock to the laboratory. Usually, the pasture sample is then dried and ground, before being sent to a commercial laboratory, for either near-infrared reflectance spectroscopy (NIRS) or wet chemistry. These results are presented as a nutritive value profile, typically comprising metabolisable energy (ME), soluble sugars (SolSug), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), non-structural carbohydrate (NSC), organic matter digestibility (OMD), crude fat (CFat), nitrogen (N) and ash.

NIRS is an accurate and cost-effective way to analyse pasture nutritive values, especially when larger sample sets are being analysed (Corson *et al*. 1999). During NIRS, a sample is assessed in terms of its absorption properties in the near-infrared electromagnetic region, which depends on the chemical composition of the sample (Corson *et al*. 1999; Deaville & Flinn 2000; Alomar *et al.* 2003).

Changes in the chemical composition of the sample between harvest and laboratory analysis may result in an inaccurate estimate of nutritive value. A post-sampling procedure (i.e. transportation, storage and drying before laboratory analyses) may be simple, where a harvested pasture sample is transported from the paddock to the laboratory in a chilly-bin with ice-packs, placed directly into a 65°C oven for 48 hours and then ground. Alternatively, a post-sampling procedure may be intensive where a cut pasture sample is snap-frozen in the paddock using liquid nitrogen and on return to the laboratory, stored at -80°C until the sample is freeze-dried and ground.

The latter method should give the most accurate estimates of nutritive value as the pasture sample remains frozen from the time it is cut in the field until after freeze-drying. The rapid freezing in liquid nitrogen halts plant respiration instantly, when compared with conventional freezing at -18°C, giving less cell disruption and protein denaturation (Alomar *et al.* 2003). In comparison to oven-drying, freeze-drying also results in less changes to the protein structure (Alomar *et al.* 2003). Presumably, these methods would better preserve key nutritive value attributes such as metabolisable energy and soluble sugars. However, an intensive post-sampling procedure may not always be achievable; there may be budget limitations and other constraints to accessing liquid nitrogen and freeze-driers, especially in remote rural areas.

This study compared post-sampling procedures with different combinations of transportation from the field to laboratory, storage methods in the laboratory and drying methods before commercial analyses. It was envisaged that results would act as a guide for choosing

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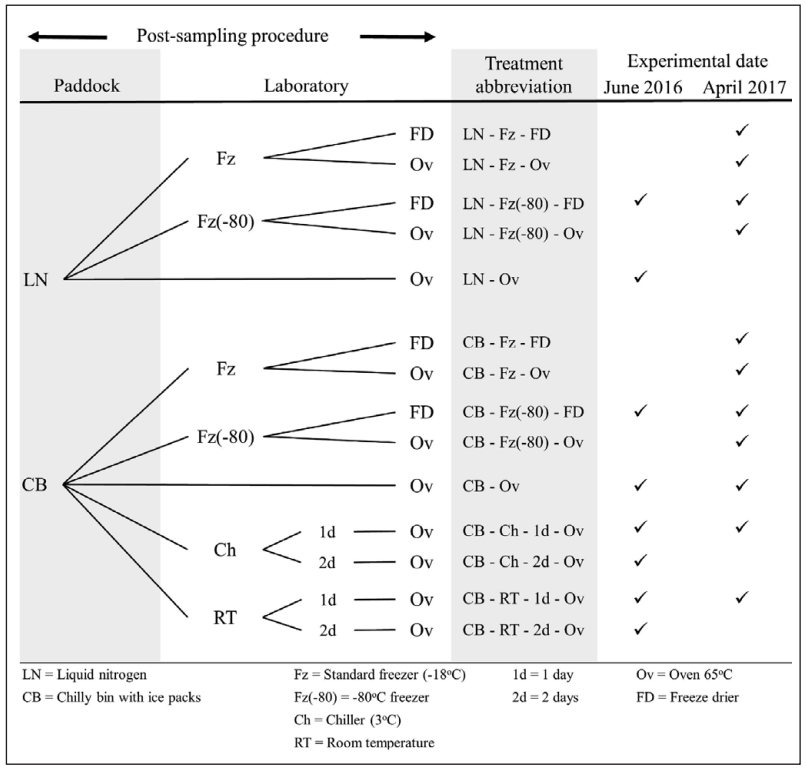

Figure 1 Summary of the fourteen post-sampling procedures (treatments) for pasture samples collected in June 2016 and April 2017. There were eight treatments for June 2016 and eleven treatments for April 2017, with five treatments overlapping for both sampling dates. Each treatment is a different combination of transportation from the paddock to the laboratory, storage in the laboratory and drying method.

the best practice post-sampling procedure, based on the equipment and budget available and the requirement for accurate nutritive value estimates.

Materials and methods

Pasture sampled

Two perennial ryegrass cultivars were selected from an agronomic evaluation located near Morrinsville, Waikato, New Zealand. 'Trojan' (diploid cultivar) and 'Bealey' (tetraploid cultivar) were chosen because of their similar genetic background and both were infected with the endophyte NEA2. Pastures were 1-year old at the time of the first sampling. At the time of sampling, ryegrass comprised 90% of total ground cover in 2016 and 70% in 2017 (averaged over all plots). Unsown species were predominantly weedy grasses, with summer grass (*Digitaria sanguinalis*) the most prevalent.

Experimental design

Ryegrass plots were arranged in a randomised complete block, with four replicate plots (each 2.4 x 5 m) for each cultivar.

Sampling

The first sampling occurred in June 2016 and the second in April 2017. Pasture samples were collected within 2 hours of sunrise for both sampling dates, to limit the effect of diurnal variation in water soluble carbohydrates on sampling (Fulkerson *et al.* 1994). In June 2016 samples were also collected 2 hours before sunset. There was no afternoon sampling in April 2017. Within each plot, approximately 1 kg of pasture was harvested to grazing height (4 cm) with electric hand-shears (Heiniger 12V handpiece). For each plot the pasture was mixed thoroughly and approximately 100 g subsampled for each subsequent treatment.

Post-sampling treatments

The post-sampling treatments differed in method of transport, storage conditions in the laboratory and drying method. Not all possible combinations were tested in all years. Figure 1 outlines the treatment combinations investigated.

Transportation from the paddock

Two methods were used to transport the samples from the paddock to the laboratory: (1) liquid nitrogen (-196˚C) and (2) chilly-bin with ice-packs (10-15˚C). For those transported at -196˚C, the 100 g subsamples were first placed in perforated plastic bread bags, immersed in liquid nitrogen to snap-freeze the sample, before transportation to the laboratory in a polystyrene box containing liquid nitrogen. The samples transported at 10˚C were placed in a large plastic bag before transporting to the laboratory in a chilly-bin with ice-packs, where the 100 g subsamples were transferred to perforated bread bags. Transportation time from the paddock to the laboratory was 40 minutes.

Storage at the laboratory

Once the samples reached the laboratory they were stored in one of the following ways: in a 3°C chiller for 1 or 2 days, on the laboratory bench at room temperature (19°C) for either 1 or 2 days, in a standard freezer (-18°C) for 10 days, or in a -80°C freezer for 10 days. Other sub-samples were not stored but oven-dried immediately after they were received from the paddock.

Drying and grinding

Two methods were used to dry the samples; freeze-drying and oven-drying. The freeze-drying process began with samples being frozen at -30°C for 18 hours. The vacuum in the freeze-drier was then increased to 100 mbar, after which samples were dried at 10°C for 6 hours followed by 0°C for 72 hours and finally 10°C for 6 hours before the vacuum was released. Samples for oven-drying were placed in a forced-draught oven for 48 hours at 65°C.

Dried pasture samples from the June 2016 sampling were ground to <1 mm at AgResearch, Ruakura, using a laboratory mill. They were then stored in an airtight container at room temperature until being taken to Hill Laboratories for analyses. Samples harvested in April 2017 were sent directly to Hill Laboratories where they were milled to <1 mm particle size.

Nutritive value tests

All results were reported on a dry matter (DM) basis using a range of methods and calibrations against *in vivo* trials. Calibration was built individually for each sample analysed, with 5-10% of samples analysed via wet chemistry reference methods to ensure robust calibration (Hill Laboratories). Crude protein (CP) was calculated from nitrogen content (N) x 6.25; metabolisable energy (ME) was calculated from dry organic matter digestibility (DOMD) x 0.16 and non-structural carbohydrate (NSC) was calculated from 100-(CP, ash, crude fat (Cfat) and neutral detergent fibre (NDF)) (http://www.hill-laboratories.com/file/fileid/44350).

Statistical analysis

Data were analysed by split-plot analysis of variance using GenStat, 18th edition (GenStat 2015). Factors fitted in the analyses were two cultivars (diploid, tetraploid), two times of day (AM, PM (first year only)), eight post-sampling treatments in the first year or eleven post-sampling treatments in the second year and all interactions. Each year was analysed separately. Cultivar and time of day was included in the analysis at the main plot level, and treatment at the sub-plot level. Comparison of individual treatments with the control (liquid N_2, -80°C freezer, freeze-drier) and other single treatment comparisons of interest were assessed by Fisher's protected least significant difference (LSD). Orthogonal contrasts were used to test the effect of liquid nitrogen versus chilly-bin in each year, room temperature versus chiller in the first year, and standard freezer versus -80°C freezer and freeze-drying versus oven-drying in the second year.

Results

Comparison of treatments

For the eight June 2016 treatments and the eleven April 2017 treatments there were significant differences for ME, SolSug, OMD, NDF, ash, Cfat ($P<0.001$) and NSC ($P<0.01$) (Figures 2 and 3 (data not presented for CFat which showed few treatment effects)). There were no differences between treatments for CP and ADF ($P>0.05$) (data not presented). Results were generally similar for both sampling dates.

Transportation of samples

Effect of liquid nitrogen versus chilly-bin, June 2016: ME and OMD were higher, and Cfat lower, when transported in liquid nitrogen than in a chilly bin (liquid N_2, oven-dried versus chilly-bin, oven-dried) (Figure 2, $P<0.05$). *April 2017*: ME, SolSug, OMD and NSC were higher, and NDF was lower, for samples transported to the laboratory in liquid nitrogen than in a chilly-bin (Figure 3, $P<0.05$). For example, SolSug was 10% lower and NSC 5% lower for chilly-bin, -80°C freezer, freeze-dried samples than the control (liquid N_2, -80°C freezer, freeze-drier) (Figure 3, $P<0.05$). Overall, samples transported in liquid nitrogen showed least change in nutritive value.

Sample storage

Effect of standard freezer (-18°C) versus -80°C freezer, April 2017: There were no differences between the standard freezer and -80°C freezer in ME, SolSug, OMD or NDF ($P>0.05$). However, NSC was higher, and ash lower, for samples stored in the standard freezer than in the -80°C freezer (Figure 3, $P<0.05$).

Effect of chiller versus room temperature, June 2016: NDF was higher, and ash lower, for samples stored in the chiller than at room temperature (Figure 2, $P<0.05$). *April 2017*: ME, SolSug and OMD were higher, and ash was lower, for samples stored in the chiller than at room temperature (Figure 3, $P<0.05$). Results were not consistent between samplings dates, but overall, samples stored at room temperature gave the least accurate estimates of nutritive value. As a general trend across all of the post-sampling procedures, samples stored in the chiller or at room temperature gave the lowest ME, SolSug, OMD and NSC and the highest NDF and ash (Figures 2 and 3).

Effect of freezer versus non-freezer storage methods, April 2017: ME, SolSug and OMD were higher, and ash was lower for samples stored in a freezer (either -80°C

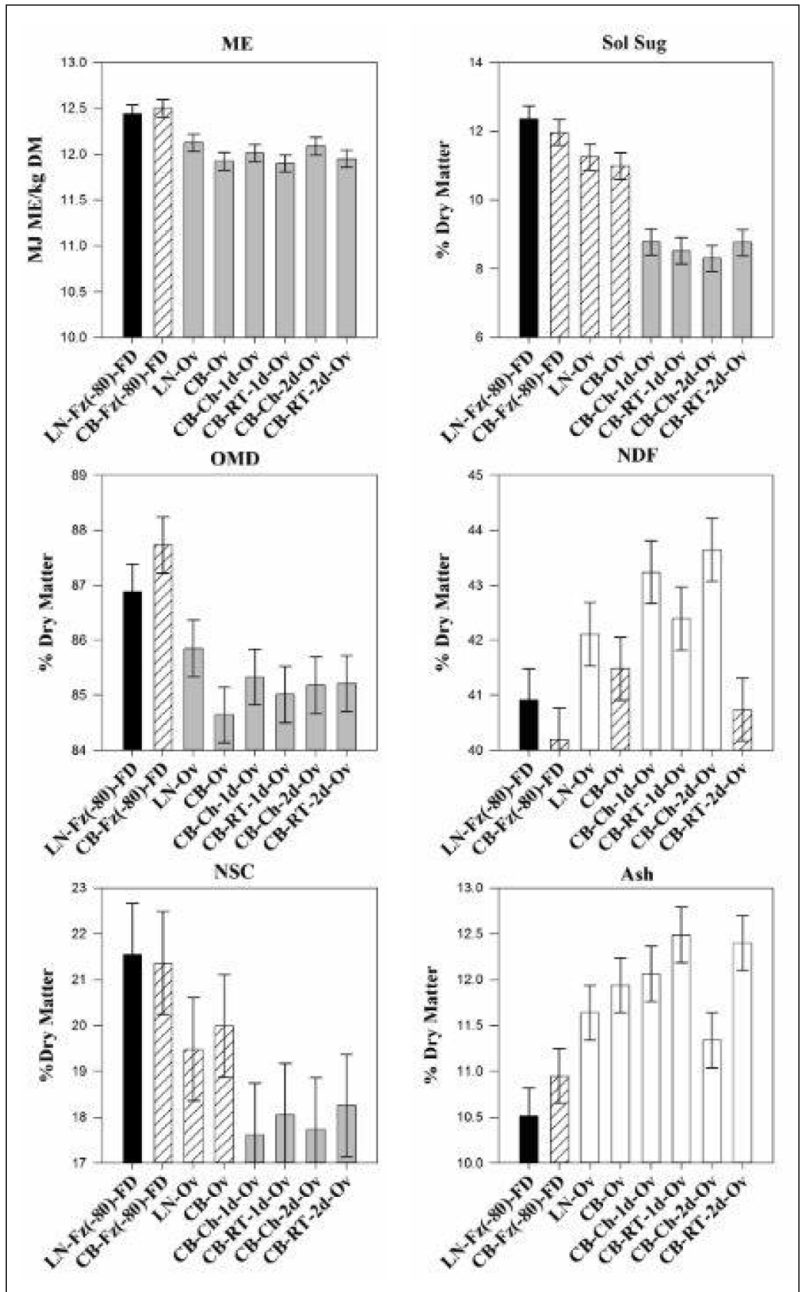

Figure 2 Metabolisable energy (ME), soluble sugars (SolSug), organic matter digestibility (OMD), neutral detergent fibre (NDF), non-structural carbohydrate (NSC), and ash values are presented for eight post-sampling methods, sampled in the morning for the mean of a diploid and tetraploid perennial ryegrass in June 2016. Shading indicates if a nutritive value was significantly lower, higher or similar to the control (liquid N_2, -80°C freezer, freeze-dryer) ($P<0.05$). Black bars: control; grey bars: lower than the control; white bars: higher than the control; and hatched bars: no difference to the control. LN, liquid nitrogen; CB, chilly-bin; Fz, standard freezer (-18°C); Fz, (-80), -80°C freezer; 1d, 1 day; 2d, 2 days; RT, room temperature (19°C); Ch, chiller (3°C); Ov, oven-dry (65°C); FD, freeze-dry. Bars are the standard errors of the mean.

or standard freezer) than in the chiller or at room temperature (Figure 3, $P<0.05$). Overall, samples stored in a freezer showed the least change in nutritive value.

Drying method

Effect of freeze-drying versus oven-drying, April 2017: ME, SolSug, OMD and NSC were higher, and NDF was lower, for freeze-dried than oven-dried samples ($P<0.001$). For example, nutritive values were lower for liquid N_2, -80°C freezer, oven-dried samples than the control (liquid N_2, -80°C freezer, freeze-drier) by 3% for ME, 11% for SolSug, 2% for OMD and 8% for NSC (Figure 3, $P<0.05$).

Cultivar

In June 2016, differences between the diploid and tetraploid cultivar were detected for ME, SolSug, OMD, and NDF (data not presented, $P<0.05$). There was an interaction between cultivar and sampling method for SolSug, but there were no consistent trends in how the different post-harvest sampling methods affected the diploid and tetraploid SolSug values. By April 2017, the only difference between the two cultivars was in SolSug (being 18% greater in the tetraploid than diploid cultivar ($P<0.05$)) and there was no interaction between cultivar and sampling method ($P>0.05$).

Sampling time (June 2016 only) ME, SolSug, OMD and NSC were higher, and NDF and ash

lower, in the afternoon than morning sampling (data not presented, $P<0.05$). For example, in the control treatment (liquid N_2, -80°C freezer, freeze dryer), nutritive values were higher from afternoon than morning sampling by 11% for SolSug, 14% for NSC, and lower from afternoon than morning sampling by 3% for NDF. There was an interaction between sampling time and post-harvest sampling method for ME, SolSug, OMD, NDF, NSC and ash (data not presented, $P<0.05$). However, results showed no consistent trends and were not investigated further in this study.

Discussion

Post-sampling procedure affects nutritive value estimates of perennial ryegrass-based pastures. Choice of drying method had the greatest impact on preserving nutritive attributes. There were large differences between freeze-drying and oven-drying, with higher values for ME, SolSug, OMD, NSC and a lower value for NDF for freeze-dried samples, regardless of cultivar. Similar trends were reported by Alomar *et al*. (2003) for DOMD and NDF in pasture samples. Kohn & Allen (1992) suggest insoluble compounds precipitate or undergo structural changes during oven-drying, which may explain why samples dried at 65°C had lower nutritive values. In comparison with freeze-drying, freezing and transporting pasture samples in liquid nitrogen had

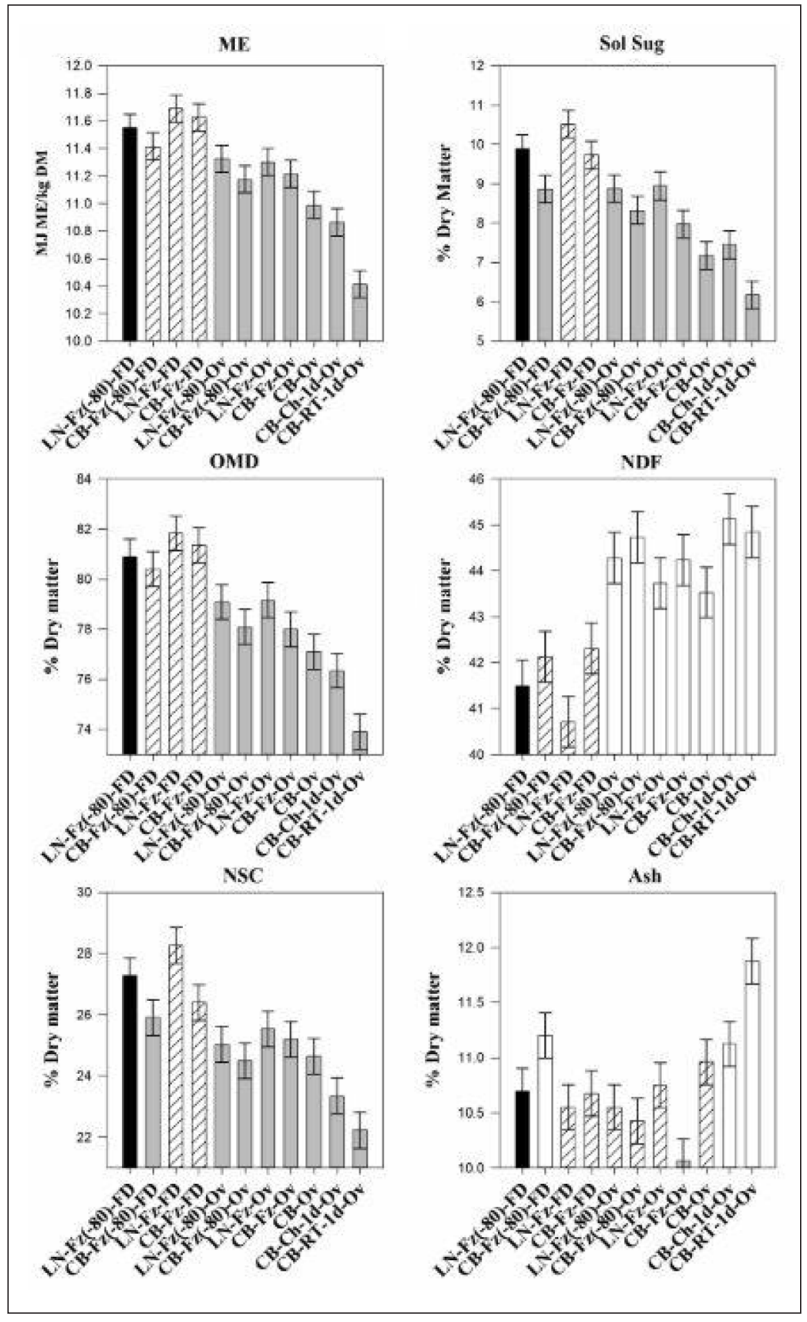

Figure 3 Metabolisable energy (ME), soluble sugars (SolSug), organic matter digestibility (OMD), neutral detergent fibre (NDF), non-structural carbohydrate (NSC), and ash values are presented for eleven post-sampling methods, sampled in the morning for the mean of a diploid and tetraploid perennial ryegrass in April 2017. Shading indicates if a nutritive value was significantly lower, higher or similar to the control (liquid N_2, -80°C freezer, freeze-drier) ($P<0.05$). Black bars: control; grey bars: lower than the control; white bars: higher than the control; and hatched bars: no difference to the control. LN, liquid nitrogen; CB, chilly-bin; Fz, standard freezer (-18°C); Fz (-80), -80°C freezer; 1d, 1 day; 2d, 2 days; RT, room temperature (19°C); Ch, chiller (3°C); Ov, oven-dry (65°C); FD, freeze dry. Bars are the standard errors of the mean.

a smaller effect on preserving nutritive value, but prevented sample alteration and a loss of nutritive value more so than transporting samples in a chilly-bin. This is likely due to liquid nitrogen rapidly reducing plant metabolic activity i.e. respiration and preserving macromolecular structures (Pelletier *et al.* 2010).

Freezer temperature had less impact on nutritive value than sample transportation or drying method, with little difference between storage at -80°C or in a standard freezer, except for NSC and ash. Storing samples in a freezer better preserved nutritive value attributes than storage in the chiller or at room temperature. Across all the post-sampling procedures, nutritive value preservation was poorest for chiller and room temperature stored samples. When comparing chiller stored samples to room temperature stored samples, the chiller best preserved nutritive attributes but results were inconsistent between years; and may reflect the difficulty in controlling these storage environments. Dale *et al.* (2016) compared storage of pasture samples in a fridge at 4°C versus ambient temperatures and concluded samples were best stored in a fridge and analysed within 24 hours of sampling.

Access to liquid nitrogen and a freeze-drier to obtain accurate estimates of nutritive value may be limited to those undertaking scientific research; an underestimate of nutritive value may therefore be inevitable for more basic sampling practices. However, it is often the relative nutritive values of forage samples that are required rather than an absolute estimate, for example, when ranking the nutritive values of different ryegrasses or other forage species. If this is the case, consistency in post-harvest sampling method is important. Results from this study show that the different post-harvest sampling methods gave similar rankings for nutritive value in both years, demonstrating that the different methods can give consistent results. When a particular method is chosen, consistency in other factors, such as time of day of sampling, are also important and will enable nutritive value estimates to be more easily compared.

Conclusions

When transporting, storing and drying pasture samples post-harvest, the following is recommended:

- Snap-freeze and transport the samples in liquid nitrogen to the laboratory as quickly as possible; transporting samples in a chilly-bin will result in loss of nutritive value.
- In the laboratory, store the samples in a standard freezer (-18°C) or in a -80°C freezer, preferably not in a chiller or at room temperature.
- For an accurate estimate of nutritive value, the most important factor is to freeze-dry the pasture samples, oven-drying will result in a loss of nutritive value.
- If there is no access to liquid nitrogen or a freeze-drier, then consistency of post-sampling procedure is important, as is processing the samples as quickly as possible. For example, harvest the samples at the same time of day if sampling on different days. Avoid the samples being in transit, stored at room temperature, or in the fridge (chiller) for several days.

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Implementing change: barriers and opportunities

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Abstract

Severe flooding and slips in the Manawatu-Whanganui region in 2004 resulted in the implementation of the voluntary Sustainable Land Use Initiative (SLUI), to support recovery and increase resilience. This paper identifies the barriers, opportunities and lessons for the implementation of Whole Farm Plans (WFPs). The research, conducted in 2016, involved interviews with 40 farm households. The findings demonstrate that the values and priorities of both SLUI and non-SLUI households were similar, highlighting the need to increase profitability, productivity and environmental protection. Both adopters and non-adopters express similar concerns about SLUI, including perceptions about its bureaucratic nature, cost and complexity. They differed to an extent when believing the SLUI aligns with their own values and goals. Non-signatories highlight their resistance to government intervention. They also feared the SLUI would thwart productivity and profits. There remains a need to more explicitly integrate social values and goals in any strategy to implement Whole Farm Plans.

Keywords: Sustainable Land Use Initiative, barriers, opportunity, values, environment, resilience

Introduction

The massive storm that hit New Zealand in February 2004 had its greatest impact on the Manawatu-Whanganui region. The resultant floods constituted the largest emergency management event in New Zealand for 20 years, and the first major natural disaster to occur under new Civil Defence legislation (Ministry of Civil Defence and Emergency Management, 2004). The total cost of damage to agriculture alone was estimated at $180 million, including stock losses; interruptions to milking; loss of pasture; damage to fences, plant and equipment; silting; and loss of feed and production. In hill country, 62 000 individual landslides were recorded, covering 18 000 ha. Twenty-nine thousand hectares were severely eroded (Horizons 2004; New Zealand Press Association 2004).

The major damage in hill country was slips. Erosion had major, negative impacts on water quality. In addition, downstream, large quantities of soil were deposited reducing the protection of infrastructure and farm land provided by stop-banks. An estimated 200 million tonnes of soil were lost.

The floods reprioritised erosion control on the local Council's agenda. In response, The Sustainable Land Use Initiative (SLUI) was designed and implemented. The SLUI aimed to reduce erosion rates closer to natural levels; build resilience in the rural sector and in the regional economy; protect lowland communities from the effects of upstream hill country erosion; and improve water quality (Horizons 2007).

The SLUI was introduced in 2005. Ten years later Horizons commissioned research to explore farmers' perspectives on how the SLUI contributed to sustainability and to identify any improvements that could be made to streamline delivery (Horizons 2016).

Methods

A core data set was developed through a series of in-depth, semi-structured interviews involving 20 hill country farmers who have signed-up to SLUI, and 20 who have not. In addition, all Field Officers employed by Horizons completed a questionnaire and subsequently met as a group with the authors to discuss their views.

Two priority areas, the Lower Rangitikei and Tiraumea, were identified as the study areas. Horizons generated a random list of farmers in each area. The questionnaires were field tested and where necessary revised. All interviews were conducted, face-to-face in May-July 2016. The design and data analysis of this qualitative social research was "triangulated" a process where two or more social science methods were used, in this case interviews and literature associated with WFPs, to test the strength of views expressed.

Incentives and barriers

Each farmer, after signing-up to SLUI, met the cost of farm plans and made a contribution towards the cost of environmental work (11/20). This bracketed ratio, here and elsewhere in the text, gives the number of responses over the total possible number of respondents. These numbers are not statistically valid, given the small

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