Using NIRS to predict composition characteristics of *Lolium perenne* L. cultivars

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Introduction Near infrared absorbance spectroscopy (NIRS) has become a preferred tool for the routine analysis of agricultural products. This is due to its rapid multi-constituent analysis capability and low sample cost, once a calibration is established. The most expensive step is the development of the prediction equation and this critical stage dictates its future performance as an analytical tool. One important potential application of the NIRS system is in quantifying quality attributes of large numbers of grasses in variety evaluation programmes. This paper reports the development of new predictive calibrations for four chemical attributes of grass composition, namely buffering capacity (BC), crude protein (CP), dry matter digestibility (DMD) and water soluble carbohydrate (WSC), using data from the DAFF national grass variety evaluation programme in Ireland.

Material and methods Ryegrass samples were collected from replicated field plots used in grass variety evaluation studies over a number of years in Ireland (McGilloway, 2003). A 300g sub-sample was collected from each harvested plot (n = 4170) and dried at 80^{O}C for 17 hours. Milled (1 mm sieve) samples were scanned on a NIRsystems 6500 (Foss UK Ltd., Warrington, UK). Absorbance (log 1/reflectance) was measured every 2 nm between 400-2500 nm and the spectra stored. WinISI (Infrasoft International, Port Matilda, PA, USA) selected 695 of these samples to provide unique information for the equation using the Neighbourhood H statistic. These 695 samples were analysed for the four attributes (BC, CP, DMD and WSC) as described by McEniry *et al.* (2006). Mathematical treatment of the spectra was carried out on WinISI. Standard normal variate (SNV) and Detrend scatter correction and a 1,4,4,1 derivation were carried out on the spectra to minimise the effects of background noise. A modified partial least squares regression (MPLS) technique was used to correlate the spectral data to the laboratory values. MPLS divides the data to a small number of independent factors and correlates these factors with the reference values. To validate the equation the sample set was divided into five equal groups of 139 samples. Each of these groups was removed in turn as a validation set and an equation formed using the remaining 556. This equation was then used to predict the validation set and the NIRS predictions were compared with laboratory methods.

Results The equation formed had strong correlations between reference and NIRS predicted values (Table 1, 0.92-0.99). The validation set had slightly lower correlations for each of the constituents but still remained strong (Table 2, 0.90-0.99). The standard error of each of the constituents was slightly higher for the validation set compared to the full data set. According to Sinnaeve *et al*. (1994) an SD/SECV ratio of greater than 3.0 is acceptable for quantitative analysis, and all four composition variables analysed were greater than this value (Table 2, 3.26-11.05). The BC and DMD ratios were closest to the threshold, but the cause was not evident in the data and not associated with the range of variation, as although DMD was smallest and CP greatest, BC and DMD were similar.

Table 1 MPLS statistics of calibration for each of the four composition variables

Constituent	N	Mean	SD	SEC	R^2
Buffering capacity (mEq/kgDM)	685	386.73	62.80	17.41	0.9231
Crude protein (g/kgDM)	688	149.50	41.17	3.50	0.9928
DMD (g/kg)	695	796.82	45.43	12.57	0.9235
WSC (g/kgDM)	684	197.22	61.60	9.79	0.9747

Table 2 The cross validation statistics of each of the four composition variables

Constituent	SECV	1-VR	SD/SECV
Buffering capacity	19.41	0.9044	3.26
Crude protein	3.74	0.9917	11.05
DMD	13.10	0.9168	3.47
WSC	10.39	0.9715	6.13

Conclusion The equation described in this paper is both accurate (R^2>0.9) and robust (SD/SECV>3.0) in the quantitative analysis of dried milled *L. perenne* samples. However, reasons for differences in SD/SECV ratio between parameters merits further study. As these samples were from different cultivars of different maturity and ploidy, as well as from different years and time of season, the equation has the potential to be used on a wide range of ryegrass herbages, particularly those from the national grass variety evaluation programme in Ireland.

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