Irish Journal of Agricultural and Food Research **53:** 199–204, 2014

A note on the comparison of three near infrared reflectance spectroscopy calibration strategies for assessing herbage quality of ryegrass

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Perennial ryegrass (n = 1,836), Italian ryegrass (n = 137) and hybrid ryegrass (n = 103) herbage was taken from harvested plots from the Irish national variety evaluation scheme and analysed for *in vitro* dry matter digestibility, water soluble carbohydrate concentration, crude protein concentration and buffering capacity. Spectral data were obtained using near infrared reflectance spectroscopy and three calibration strategies (global, species-specific or local) were utilised to relate the reference values to the spectral data. The local strategy generally provided the poorest estimation of herbage composition, with global and species-specific calibration strategies producing similarly accurate estimates of each quality trait. The higher accuracy and easier maintenance of the global strategy make it the recommended calibration method for analysing quality of ryegrass.

Keywords: calibration strategy; near infrared reflectance spectroscopy; quality; ryegrass

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Introduction

Near infrared reflectance spectroscopy (NIRS) is a secondary technique for analysing herbage quality traits that uses predictive calibration models to relate spectral data to values obtained by standard laboratory procedures. Applying a combination of chemometric techniques and calibration methodologies form the basis of a calibration strategy, with no one approach being universally optimal (Givens, DeBoever and Deaville 1997; Stuth, Jama and Tolleson 2003).

A global calibration strategy involves all samples within the calibration set being utilised, with multiple linear regression models relating spectral data to reference values (Burns *et al.* 2013). The local calibration strategy of Shenk, Westerhaus and Berzaghi (1997) is potentially more accurate in calibration sets that contain several forage species, as these could introduce non-linear relationships. The current study contains three species; perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and hybrid ryegrass (*Lolium boucheanum* Kunth), that may potentially introduce a bias between species within the calibration models. A third strategy, species-specific, develops individual calibration models for each species from within the total calibration set in order to remove the bias between species. These smaller datasets can potentially lower the range within quality traits for the individual species-specific calibration models.

The objective was to ascertain the optimal NIRS calibration approach from three calibration strategies (global, species-specific and local) for assessing the *in vitro* dry matter digestibility (DMD), water soluble carbohydrate (WSC) concentration, crude protein (CP) concentration and buffering capacity of ryegrasses.

Material and Methods

Variety trials were carried out at the DAFM Variety Evaluation Centre, Backweston, Co. Kildare. These were sown in separate trials for each of the species perennial ryegrass, Italian ryegrass and hybrid ryegrass. Varietal monocultures were harvested under a six cut combined simulated grazing and conservation management schedule as described by Burns *et al.* (2013). Ryegrass samples (n = 2,076) were collected from harvested plots and selected to ensure the calibration models encompassed the range in climatic conditions, species, developmental stage, ploidy, genotype and age of sward occurring in the recommended list trials (Burns 2012).

Reference laboratory methods were carried out on each sample as described by Burns *et al*. (2013).

Absorbance (log 1/reflectance) of each sample was measured from 400 to 2,500 nm at 2 nm intervals using a NIRsystems 6,500 or a standardised NIRsystems XDS (Foss UK Ltd., Warrington, UK). Prior to development of calibrations each spectrum was trimmed to 1,108–2,498 nm at 2 nm intervals. Burns *et al*. (2013) established a standard normal variate and detrend (Barnes, Dhanoa and Lister 1989) and a 1,4,4 derivation as spectral pre-treatments that produced the most accurate calibration models and these were applied to each spectrum using WinISI v. 4.00 (Infrasoft International, Port Matilda, PA, USA).

Three calibration strategies were applied to relate spectral data to the reference values:

(a) Global strategy – The global strategy of Burns *et al.* (2013) was used, in which the full calibration set (n = 2,076) was utilised to form calibration models for each of the four quality traits.

(b) Species-specific strategy – For the species-specific strategy the full calibration

set (n = 2,076) was divided into a subset for each individual species: perennial ryegrass (n = 1,836), Italian ryegrass (n = 137) and hybrid ryegrass (n = 103). The same approach for the global calibration was applied to each of these individual subsets. A weighted mean, as weighted by the number of samples from each species-specific strategy, was calculated for the coefficient of determination (R^2) and the standard error of calibration (s.e.c.) for each of the four quality traits for both the calibration and cross-validation models.

(c) Local calibration strategy – The local algorithm of Shenk *et al.* (1997) was used in which principle component analysis was initially applied to the calibration set. Following this, an individual calibration was formed for each sample and quality trait whereby either 10, 25, 50, 100, 150, 200 or 250 samples (k) were selected based on the closeness in hyper dimensional space. Subsequently a multiple linear regression model was developed using the k samples to predict a value for the sample in question. This process was repeated for each individual sample so effectively an individual calibration set was generated for each sample.

For each of the calibration models a cross-validation was carried out in which a random four-fifths of the full calibration set was used to form the calibration models and the remaining samples were used as a validation set. The cross-validation procedure was carried out five times and the mean of the cross-validation procedures calculated.

Results

The calibration sample set displayed a wide range in each of the herbage quality traits when assessed by standard laboratory techniques. Samples ranged from *in vitro* DMD 576–890 g/kg; 50–430 g WSC/kg DM; 57–267 g CP/kg DM and buffering capacity 188–736 mEq/kg DM (Table 1).

Overall the calibration models produced were accurate, with cross-validation coefficient of determination (R^2_{cv}) above 0.94 in all cases, with the exception of *in vitro* DMD for a global strategy (R^2_{cv} = 0.86), local strategy (R^2_{cv} = 0.80,

Table 1. Summary of the values obtained by standard laboratory techniques for four quality traits of three ryegrass species

	n	Mean	Minimum	Maximum	s.d.
In vitro dry matter digestibility (g/kg)					
Perennial ryegrass	1,836	801	629	890	36.9
Italian ryegrass	137	748	576	870	73.7
Hybrid ryegrass	103	773	587	886	70.7
Water-soluble carbohydrate (g/kg DM)					
Perennial ryegrass	1,836	180	50	376	51.9
Italian ryegrass	137	207	92	430	74.4
Hybrid ryegrass	103	212	71	413	70.4
Crude protein (g/kg DM)					
Perennial ryegrass	1,836	150	57	266	36.8
Italian ryegrass	137	128	73	267	40.5
Hybrid ryegrass	103	133	71	241	43.4
Buffering capacity (mEq/kg DM)					
Perennial ryegrass	1,836	437	215	736	90.8
Italian ryegrass	137	360	188	559	87.5
Hybrid ryegrass	103	368	207	616	83.6

k = 200) and species-specific strategy for perennial ryegrass (R^2_{cv} = 0.78), and the weighted mean for the species-specific strategy (R^2_{cv} = 0.80) (Table 2). The global and species-specific calibration strategies produced similarly accurate estimates of each quality trait. Comparing individual species sets within the species-specific strategy, the accuracy of estimations was relatively similar except for the *in vitro* DMD estimation in perennial ryegrass, which was the weakest estimation. Applying the local strategy generally provided the poorest estimation of the three strategies, with *in vitro* DMD having the weakest estimates (R^2_{cv} = 0.80). The R^2_{cv} of the local strategy for WSC, CP and buffering capacity accuracy approached the levels of the other two strategies, but never exceeded them.

Discussion

A global strategy uses all samples from the calibration set and should encompass as much of the variability as possible that the model will likely face in practice (Shenk and Westerhaus 1991). The current study had a large calibration set (n = 2,076) that contained three species and additional sources of variation (e.g., age of sward, seasonal developmental stage, genotype) that encompassed much of the variation the model will likely face in practise.

Berzaghi, Shenk and Westerhaus (2000) and Andueza *et al.* (2011) reported the increased accuracy of a local calibration strategy in comparison to a global strategy for predicting herbage quality traits in calibration sets that encompassed several forage species. The greater accuracy of the global strategy in comparison to the local strategy in the current study may be attributed to the lack of bias and non-linear relationships that existed between monocultures of three species within the same genus (*Lolium*), whereas Berzaghi *et al.* (2000) and Andueza *et al.* (2011) had a larger range of diverse forages (including ryegrass, red clover, lucerne, corn silage, haylage, small grain silage and total mixed ration) which could have potentially limited the performance of their global calibration models. The use of a global strategy may therefore be more appropriate when most variation is encompassed with little bias between factors in the model.

The accuracy of the species-specific strategy for Italian and hybrid ryegrasses was higher than the species-specific strategy for perennial ryegrass or the global strategy, and both Berzaghi *et al.* (2000) and Andueza *et al.* (2011) also found that a species-specific strategy produced the most accurate calibration models for some individual forage species. It is evident that the inclusion of the Italian and hybrid ryegrass (i.e., global strategy) to the species specific approach for perennial ryegrass expands the range and distribution of *in vitro* DMD values (Table 1). This could potentially account for the increased accuracy of the global strategy in comparison to the perennial ryegrass species-specific calibration model. A comparable outcome occurred for the standard deviation of reference values with WSC, whereby there was a lower standard deviation for perennial ryegrass samples than for both other species; however the resultant global and species-specific strategies were of similar accuracy. The range and distribution of reference values for CP and buffering capacity between species was quite similar (Table 1) and this may have led to the similar accuracy of the species-specific and global calibration strategies.

When considered across all species, a global or species-specific calibration strategy produced the most accurate calibration models for WSC and CP, while for *in vitro* DMD the global strategy was the

Table 2. Comparison of three calibration strategies (Global; Local; Species-specific) for the development of NIRS predictive models for the analysis of four quality traits

		In vitro DMD (g/kg)				WSC (g/kg DM)				Crude protein (g/kg DM)				Buffering capacity (mEq/kg DM)			
		Calibration		Cross-validation		Calibration		Cross-validation		Calibration		Cross-validation		Calibration		Cross-validation	
Calibration strategy	N	R^2	s.e.c.	R^2cv	s.e.c.v.	R^2	s.e.c.	R^2cv	s.e.c.v.	R^2	s.e.c.	R^2cv	s.e.c.v.	R^2	s.e.c.	R^2cv	s.e.c.v.
(a) Global	2,076	0.864	15.9	0.858	16.2	0.961	10.4	0.959	10.8	0.982	5.0	0.981	5.1	0.952	20.3	0.950	20.7
(b) Species-specific																	
Perennial	1,836	0.788	16.0	0.782	16.3	0.961	10.2	0.959	10.5	0.980	5.1	0.979	5.3	0.953	19.7	0.950	20.3
Italian	137	0.972	11.8	0.963	13.6	0.983	9.0	0.974	11.2	0.989	4.2	0.984	5.0	0.962	17.1	0.952	19.1
Hybrid	103	0.981	9.5	0.969	12.2	0.982	9.2	0.969	11.9	0.992	3.9	0.988	4.6	0.945	19.9	0.912	24.4
Weighted mean		0.810	15.4	0.803	15.9	0.963	10.1	0.960	10.6	0.981	5.0	0.979	5.2	0.953	19.5	0.948	20.4
(c) Local																	
k = 10		0.710	24.8	0.700	25.9	0.893	18.1	0.858	21.0	0.946	8.8	0.924	10.5	0.867	34.1	0.845	34.1
25		0.739	23.4	0.715	25.2	0.917	16.0	0.904	17.4	0.960	7.6	0.945	8.9	0.917	26.9	0.898	26.9
50		0.759	22.5	0.763	22.9	0.929	14.8	0.923	15.5	0.966	7.0	0.955	8.0	0.935	23.8	0.931	23.8
100		0.785	21.3	0.796	21.3	0.934	14.3	0.931	14.6	0.969	6.7	0.959	7.6	0.949	21.0	0.943	21.1
150		0.791	21.0	0.799	21.1	0.934	14.3	0.934	14.3	0.970	6.6	0.958	7.6	0.952	20.4	0.940	20.4
200		0.792	21.0	0.803	20.8	0.933	14.4	0.933	14.5	0.968	6.8	0.958	7.6	0.953	20.4	0.943	20.2
250		0.789	21.1	0.805	21.1	0.931	14.6	0.931	14.7	0.966	7.0	0.958	7.7	0.952	20.4	0.944	20.4

s.e.c.: Standard error of calibration; s.e.c.v.; Standard error of cross-validation.

most accurate strategy and for buffering capacity all three strategies were similarity accurate. It is thus recommended that due to the higher accuracy and easier maintenance of the global strategy it would be the preferred approach when evaluating herbage quality of ryegrass samples.

Acknowledgements

The Department of Agriculture, Food and the Marine research stimulus fund (07 526) is acknowledged for providing the resources to carry out this research; the laboratory staff at Teagasc, Grange for carrying out the laboratory analyses and the field staff at the Variety Evaluation Unit, Backweston for the harvesting and management of ryegrass plots.

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Received 8 January 2013