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RESEARCH PAPER

Calibration models for the nutritional quality of fresh pastures by near-infrared reflectance spectroscopy

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Abstract

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High levels of animal performance and health depend on high-quality nutrition. Determining forage quality both reliably and quickly is essential for improving animal production. The present study describes the use of near infrared reflectance spectroscopy (NIRS) for the quantification of nutritional quality (dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), *in vitro* dry matter digestibility (DMD), organic matter digestibility (OMD), neutral detergent fiber (NDF) and the WSC/CP ratio) in samples from fresh pastures in southern Chile (39° to 40° S). Calibration models were developed with wet chemistry and NIRS spectral data using partial least squares regression (PLSR). The coefficients of determination in the validation set ranged between 0.69 and 0.93, and the error of prediction varied from 0.064 to 2.89. The evaluation of the model confirmed the high predictive ability of NIRS for DM and CP and its low predictive ability for DMD, OMD, NDF and the WSC/CP ratio. It was not possible to obtain a model for WSC because it would have required an increased number of samples to improve the spectral variability and the R² value (> 80%).

Key words: calibration models, external validation, forage, NIRS, nutritive value.

Introduction

Animal production in southern Chile relies mainly on forages and pastures that are heterogeneous in terms of species, yield and management. Grassland classification of the principal dairy and beef production systems are divided into sown (11.0%) and naturalized with (46.3%) and without management (42.5%) (Moscoso and Urrutia, 2017).

In terms of dairy and beef production, 73% of the milk produced in Chile and 44% of bovines come from the southern regions: Los Rios and Los Lagos (ODEPA, 2015). This highlights the importance of pastures and their utilization, with an inverse relationship between the use of pastures and production cost (Dillon *et al.*, 2005). It is therefore of utmost importance to know the nutritive value of forages to improve grazing management decisions (Corson *et al.*, 1999).

including the chemical composition of forages, intake and growth rates (Coates, 2000; Stuth and Tolleson, 2000). They are also becoming more commonly used to identify animal species, gender and pregnancy (Tolleson and Stuth, 2002).

The traditional characterization of the nutritional quality of forages through wet chemistry procedures has the disadvantage of being slow and destructive, requiring specialized equipment, which is usually expensive (Deaville and Flinn, 2000). In contrast, using near infrared spectroscopy (NIRS) to predict the nutritive quality of forages could prove to be a quick and effective tool for nutrient composition determination of forages, which would help improve the strategic use of supplements and adjustments in ration formulations for dairy and beef bovines.

The NIR region is the wavelength range between 700-2500 nm in the electromagnetic spectrum. When a sample is analyzed, the radiant energy is absorbed selectively according to the specific vibration of the molecule, which produces an overtone in the spectrum. Prominent absorptions in NIRS forage spectra include water, aliphatic hydrocarbons, lipids and carbohydrates (Conzen, 2006). Unlike most conventional analytical methods, NIRS is fast and nondestructive; it does not use chemicals, does not generate chemical wastes that require disposal and is also multiparametric, provided that several parameters can be determined simultaneously in the same measurement (Eldin, 2011).

There are a number of major steps that are essential to obtain a satisfactory working calibration: sample selection, acquisition of spectra and reference data, pretreatment of spectral data, derivation of the regression model and validation of the model (Deaville and Flinn, 2000). The quality of the reference-method analysis has a crucial effect on the accuracy of NIR calibrations (Conzen, 2006).

This technology is broadly accepted as a fast and reliable method for evaluating the nutritional qual-

ity of pasture silages (Ibáñez and Alomar, 2008; Restaino *et al.*, 2009), as well as dried and fresh pastures (Cozzolino and Labandera, 2002; Alomar *et al.*, 2009; Burns *et al.*, 2013; Lobos-Ortega *et al.*, 2013; Moscoso and Balocchi, 2016). It has also been used to evaluate how forage sample preparation affects nutrient composition analysis (Alomar *et al.*, 2003), green forage intake and digestibility in ruminants (Decruyenaere *et al.*, 2009), and water-soluble carbohydrates (WSC) in stolon samples of white clover (Inostroza *et al.*, 2017). NIRS has also been used to characterize and quantify isoflavones and phenolic acid contents in red and white clover (Krähmer *et al.*, 2013).

Although NIRS is a quick and relatively low-cost technology, prediction equations for assessing nutritional quality have mostly been developed using leave-one-out cross validation (Garcia and Cozzolino, 2006; Ibáñez and Alomar, 2008; Alomar *et al.*, 2009; Restaino *et al.*, 2009; Burns *et al.*, 2013). These equations do not typically utilize a independent test set to generate a robust model to predict an unknown pasture sample.

The objective of this study was to generate NIRS prediction models as a quick and effective tool to quantify the nutritional quality of fresh permanent pastures for dairy and beef production systems in southern Chile.

Materials and methods

The present study used 915 permanent pasture samples collected randomly between 2014 and 2016 from several farms located in southern Chile (39° to 40° S). These samples were grown on different soil types and under different soil fertility conditions, representing the variability of pastures used in dairy and beef production systems under grazing. Permanent pasture samples comprised different proportions of grasses, such as *Lolium perenne* L., *Agrostis* spp., *Holcus lanatus* L., *Bromus* spp., *Dactylis glomerata* L. and clovers (mostly *Trifolium repens* L. and *T. pratense* L.).

NIR Spectroscopy

Fresh samples were cut to 2-3 cm with hand shears. After this procedure, each fresh subsample was exposed to an electromagnetic scan over a spectral wavelength range of 700-2,500 nm (near infrared) using an MPA model FT-NIR (Bruker Optik GmbH, Germany). Energy in this spectral range was directed into the sample, and the reflected energy (R) was measured by the instrument. Each spectral measurement was obtained from 32 scans performed at a wavenumber resolution of 16 cm⁻¹.

Chemical analysis

At the laboratory of Nutrición Animal y Medio Ambiente (INIA Remehue), all samples were thoroughly homogenized, oven-dried at 60°C for 48 h, and ground through a 1 mm sieve, after which their chemical composition was determined. Samples were analyzed for DM (method 934.01) and CP (method 984.13), according to the procedures outlined by AOAC (1995), and WSC according to MAFF (1986). *In vitro* dry matter digestibility (DMD) and organic dry matter digestibility (OMD) were determined by incubating the samples in a two-stage rumen liquid for 48 h at 39°C, followed by incubation in a pepsin acid medium for 24 h (Tilley and Terry, 1963). Finally, NDF was determined as described by Mertens (2002).

Chemometric analysis

Partial least-squares regression (PLSR) with a test-set validation was used to calibrate the spectral data with the wet chemistry data. The PLSR algorithm selected the successive orthogonal factors that maximized the covariance between predictor (spectra) and response variables (laboratory data). The software OPUS™ version 6.0 (Bruker Optik GmbH, Germany) was used to create models by selecting wavelengths, mathematical pretreatments, PLSR factors, outlier determinations and PLSR regression, among others.

To develop and validate the regression models for each parameter, the samples were divided into two sets: a calibration set for modeling and a validation set for the developed model. To define the two groups, the samples were arranged according to the experimental value of each parameter and subsequently distributed alternately, for example, i) when the number of samples was more than 200, one sample was used for calibration and one for validation; ii) when the number of samples was less than 200, two were used for calibration and one for validation. In either case, both sets covered the whole range of chemical data (Conzen, 2006).

The PLSR method was performed on the spectral region, setting the upper limit at 10 PLS factors (Conzen, 2006). To optimize calibration accuracy, spectral data were subjected to a variety of preprocessing transformations using common scatter correction treatments and derivations of the original spectrum to transform spectral data in such a way that the signals would better adhere to Beer's law, which states that absorbance and concentration are linearly correlated (Rinnan *et al.*, 2009). The tested preprocessing transformations underwent vector normalization (VN), minimum-maximum normalization, multiplicative scatter correction (MSC), subtraction of a straight line, constant offset elimination, first and second derivative, or a combination of these options. The root mean square error of calibration (RMSEC) was used to calculate the analysis error of the calibration values (Equation 1).

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n-r-1}} \quad (1)$$

where \hat{y}_i is the NIR predictive value, y_i is the chemical reference value of sample i , n is the number of samples and r is the number of PLS factors.

The validation set was used to identify the best preprocessing technique and select the optimal number of PLS factors for each model. The root mean square error of prediction (RMSEP), which

represents an objective assessment of the overall error between modeled and reference values, was used to evaluate and compare the accuracy of the different PLS models developed (Equation 2).

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad (2)$$

where \hat{y}_i is the NIR predictive value, y_i is the chemical reference value of sample i from the prediction set of samples, and n is the number of samples.

Various PLS models were built correlating pre-processed NIRS spectral data with the chemical data of the calibration set. The best models were selected using the lowest RMSEP; therefore, we chose among the best five models the one that had the lowest number of PLS factors. The coefficient of determination of prediction (R^2_p) and the residual predictive deviation (RPD) were also used to evaluate the predictive ability of the models. The RPD is the ratio between the standard deviation (SD) of the reference values and the error of prediction; it is thus a qualitative measure for the assessment results. The smaller the error of prediction, compared to the variance of the reference values, the larger the RPD value, and

therefore, the better the model. An RPD value below 2.4 characterizes a poor model, whereas RPD values approximately 2.5-3.0 could be used for screening purposes (Williams and Sobering, 1996; Conzen, 2006).

Results and Discussion

The mean and standard deviations of DM, WSC, CP, DMD, OMD and NDF were 15.1% (± 4.10), 8.0% (± 3.13), 23.4% (± 5.57), 83.5% (± 4.97), 73.2% (± 5.66) and 44.1% (± 6.21), respectively, and the standard error of the laboratory was 0.34, 0.22, 0.32, 0.57, 0.63 and 0.90 for DM, WSC, CP, DMD, OMD and NDF, respectively (Figure 1). These mean values are similar to those found by Demanet *et al.* (2015), who studied the nutritive quality of permanent pastures over 11 years and found a range between 15.2-23.2%, 13.4-36.7%, and 48.6-58.4% for DM, CP and NDF, respectively. In another study, in the evaluation of different pasture renovation strategies, Keim *et al.* (2014) found concentrations between 58.3% and 78.3% for OMD and 5% and 12.6% for WSC, similar to those observed in the present study.

Chemical variations could be the direct result

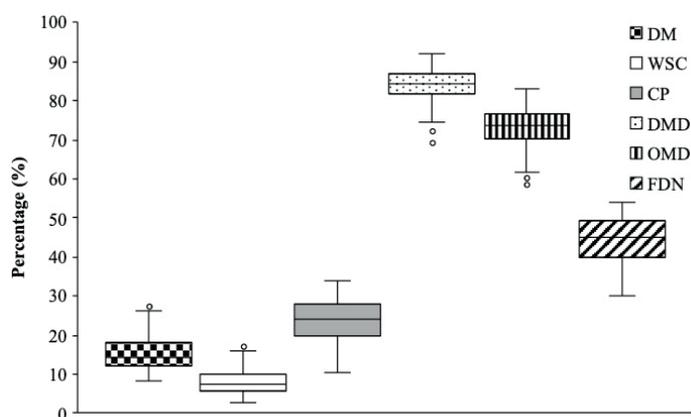


Figure 1. Boxplots displaying the mean, quartiles, outliers and the range of distribution for dry matter (DM), water-soluble carbohydrates (WSC), *in vitro* dry matter digestibility (DMD), organic matter digestibility (OMD) and neutral detergent fiber (NDF) in fresh pasture samples from southern Chile. Boxplots are the 25th, 50th and 75th percentiles, and the bottom and top whiskers are the 10th and 90th percentiles, respectively.

of variability in pasture components (botanical composition), growth stage, climatic and management factors, and soil type, among others (Hopkins, 2000). The wide range of values for nutrient composition parameters included two important conditions desirable for an optimal calibration, as described by Murray (1988): the amplitude of composition and its homogeneous distribution across the range of composition. Figure 1 shows a boxplot displaying quartiles and ranges of distribution for the nutrition quality of the sampled fresh forage.

NIRS calibration and validation

The predictive capacity of NIRS for all of the studied parameters is provided in Table 1. After testing several mathematical treatments, the best calibration was selected according to the R^2 , RMSEP and RPD statistics from the set validation. The coefficients of determination for the validation set were higher than 0.69, the errors of prediction were lower than 2.9, and RPD values above 1.8 were observed (Table 1). The relation between the NIRS prediction and the composition obtained by the reference methods is shown in Figure 2.

The calibration models obtained for DM and CP were the most robust because they obtained RPD values ≥ 2.5 , which is the critical value used for the prediction of unknown samples, and R^2 values

higher than 0.84 (Williams, 2004). On the other hand, the models generated for DMD, OMD, NDF and WSC/CP ratio were less precise (RPD ≥ 2.0), even though the R^2 and RPD parameters for NDF were greater than 0.85 and 2.6, respectively, in the calibration set (Table 1). Finally, it was not possible to obtain adequate prediction models for WSC. This may be a consequence of the high water content of the fresh pastures. The water absorption bands in the NIRS spectral data are strong and can limit the detection capacity of other constituents (Thyholt and Isaksson, 1997).

The predictive ability of these models can be explained in part by the low standard error of the laboratory given that the reference data were obtained by gravimetric and chemical analysis, techniques that present high precision in comparison to that of other techniques that include biological elements (Ibañez and Alomar, 2008).

The error of calibration for DM was lower than that obtained by Alomar *et al.* (2009), who reported values of standard error of cross validation (SECV) of 7.5, although in their study they obtained higher coefficients of determination and RPD values (0.98 and 7.15, respectively). The calibration model error obtained for CP was lower than that found by Gislum *et al.* (2004) in red fescue and perennial ryegrass and by Lobos-Ortega *et al.* (2013) and Alomar *et al.* (2009) in dry and fresh permanent pastures, respectively.

Table 1. Descriptors of the NIRS calibration and validation sets to estimate the chemical composition of fresh forage samples.

Parameters	Calibration set					Validation set				
	N	Range	R^2_c	RMSEC	RPD	N	Range	R^2_p	RMSEP	RPD
DM (%)	433	8.14-28.00	0.94	1.05	4.0	432	8.36-27.30	0.93	1.13	3.7
CP (%)	456	10.25-33.81	0.87	2.04	2.8	459	8.39-34.25	0.84	2.22	2.5
WSC (%)	144	2.80-16.60	0.74	1.68	2.0	138	2.90-17.10	0.69	1.74	1.8
DMD (%)	109	65.30-93.20	0.77	2.98	2.1	45	69.30-93.00	0.76	2.41	2.2
OMD (%)	113	56.90-86.70	0.79	3.03	2.2	53	58.60-83.00	0.78	2.61	2.2
NDF (%)	113	28.80-54.30	0.85	2.45	2.6	49	30.30-53.90	0.78	2.89	2.2
WSC/CP	100	0.01-0.73	0.76	0.06	2.0	47	0.10-0.72	0.74	0.06	2.0

DM: dry matter; CP: crude protein; WSC: water-soluble carbohydrates; DMD: *in vitro* dry matter digestibility; OMD: organic matter digestibility; NDF: neutral detergent fiber; N: number of samples; R^2_c : coefficient of determination for calibration; RMSEC: root mean square error of calibration; RPD: residual prediction deviation; RMSEP: root mean square error of prediction; R^2_p : coefficient of determination in prediction.

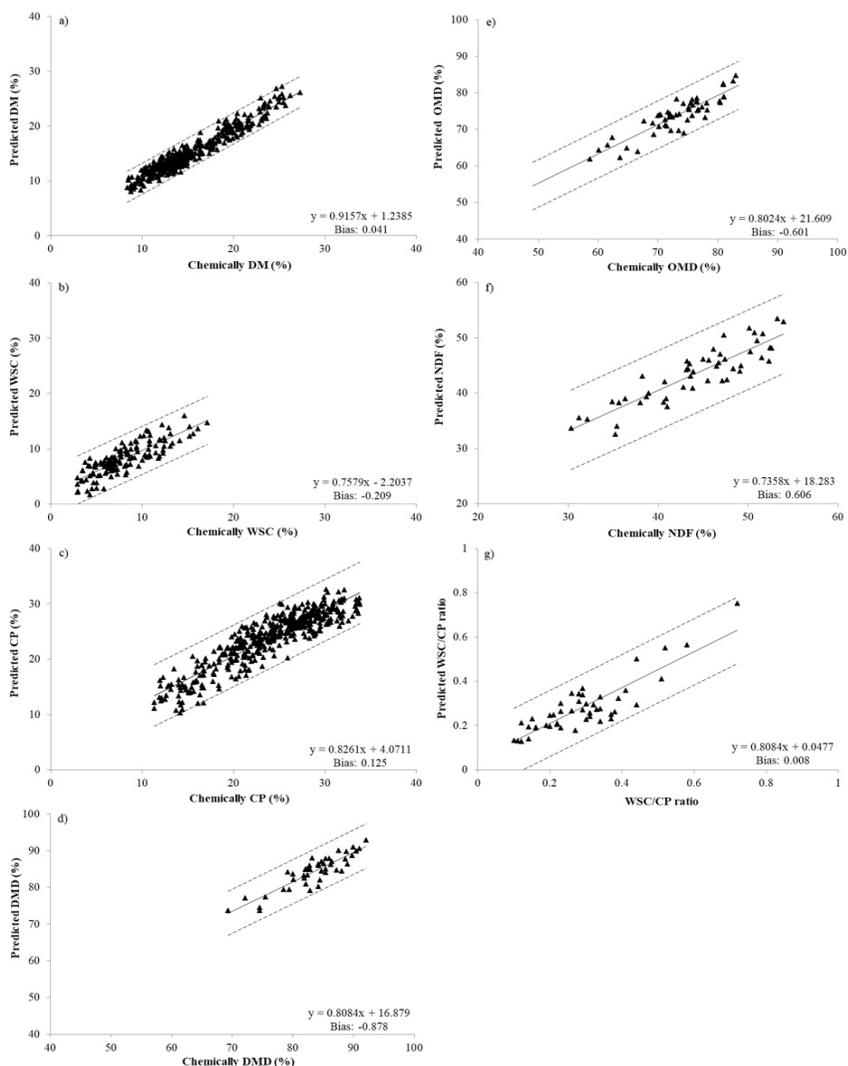


Figure 2. Correlation of wet chemistry and the predicted NIRS for a) dry matter (DM), b) water-soluble carbohydrates (WSC), c) crude protein (CP), d) *in vitro* dry matter digestibility (DMD), e) organic matter digestibility (OMD), f) neutral detergent fiber (NDF) and g) the WSC/CP ratio in fresh pasture samples from southern Chile. The diagonal line represents the equal response line.

The models generated for DMD and NDF were lower than those reported by Decruyenaere *et al.* (2009) in pastures, legumes and a forage mix and were similar to those reported by Alomar *et al.* (2009) in fresh pastures. Nonetheless, the calibration descriptors in our study were higher than those obtained by Restaino *et al.* (2009) in pasture silage. The calibration error obtained in this study for OMD was lower than that reported by Corson *et al.* (1999) in pasture samples with a similar concentration range. The coefficient of determination obtained in this study for WSC

was lower than that reported by Alomar *et al.* (2009) and Lobos-Ortega *et al.* (2014) in fresh forage samples and by Inostroza *et al.* (2017) in dried white clover. Finally, the results for R^2_c and the RPD values for the WSC/CP ratio were lower in the present study than those shown by Rivero *et al.* (2014).

The predicted DM and CP showed a strong relation with the reference values (Figure 2), as shown by the concentration of individual observations around the equal response line (Figure 2a, 2c).

For the WSC parameter, the low predictive ability is confirmed by the lower correlations among the reference value and spectrum (Figure 2b). These results suggest that the water content can often mask NIR signals and generate a limited predictive model (Reeves, 2000). Although errors are often slightly higher for components such as CP, DMD, OMD and NDF in fresh samples when compared to those of dried and ground samples, this is balanced by the ability to scan a greater number of samples in less time, thus avoiding changes in the composition due to oven-drying procedures (Alomar *et al.*, 2009). In addition, the minimum sample preparation helps to reduce the time and cost of the analysis. The RMSEP and RMSEC values are very close (Table 1), indicating that the obtained calibration model retained its predictive capability for the samples that did not belong to the calibration set (González-Sáiz *et al.*, 2007).

The main conclusions are as follows. The evaluation of a calibration model obtained for fresh forage confirmed the high predictive ability of NIRS for DM and CP, a low predic-

tive quality for DMD, OMD, NDF and WSC/CP ratio, while it was not possible to obtain an adequate prediction model for WSC. It is therefore necessary to increase the number of fresh pasture samples used for calibration to improve the spectral variability and R^2 value (>80%). These results suggest that it is possible to use NIR spectroscopy to optimize pasture management decisions for grazing high quality pastures to maximize animal production.

Acknowledgments

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Resumen

I. Lobos, C.J. Moscoso, y P. Pavez. 2019. Modelos de calibración para la cuantificación nutricional de praderas frescas mediante espectroscopía de infrarrojo cercano. Cien. Inv. Agr. 46(3): 234-242. La nutrición influye en el rendimiento y salud animal, donde la determinación rápida y confiable de la calidad del forraje es esencial para mejorar la producción animal. El presente estudio describe el uso de la espectroscopía de reflectancia del infrarrojo cercano (NIRS) para la cuantificación de la calidad nutricional en términos de materia seca (MS), proteína cruda (PC), carbohidratos solubles en agua (CHOS), digestibilidad *in vitro* de la MS (DIV), digestibilidad de la materia orgánica (DMO), fibra detergente neutro (FDN) y la relación CHOS/PC en praderas frescas del sur de Chile (39 a 40° S). Los modelos de calibración se desarrollaron entre la química húmeda y los datos espectrales NIRS usando la regresión por mínimos cuadrados parciales (PLSR). El rango de coeficientes de determinación en el conjunto de validación varió entre 0,69 y 0,93 y el error de predicción entre 0,064 y 2,89. La evaluación del modelo obtenido confirmó la alta capacidad predictiva del NIRS para MS y PC, baja capacidad predictiva para FDN, DIV, DMO y la relación CHOS/PC. No fue posible obtener un modelo para CHOS, siendo necesario aumentar el número de muestras y así mejorar la variabilidad espectral y el valor de R^2 (>80%).

Palabras clave: Calidad nutritiva, forraje, modelos de calibración, NIRS, validación externa.

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