The potential of calibration transfer for quality control of undried maize silage

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Introduction

Traditional wet chemical analysis of forages and feedstuffs for animal nutrition has been used to characterise their composition and quality, but these procedures are costly, time-consuming and sometimes hazardous. Moreover, with the continuing decline in animal product prices, it is imperative that feed costs are minimised. For these reasons there is a need for the development of methodologies to ensure that feedstuffs such as maize silage can be accurately and rapidly characterised, ensuring correct diet formulation for effective milk and beef productions.

In this vein, Near Infrared Reflectance Spectroscopy has emerged in the last 30 years as a rapid method for testing the quality and characterise composition of forages.[1] Typically forages analysed using this technique have been dried and grounded prior to scanning. However, in several studies De la Roza *et al.*[2] and Park *et al*.[3] working with grass silage, have shown that drying and grounding is unnecessary if the undried grass silage is finely comminuted and scanned in a static cup with a big product scanning surface. Attending, maize silage samples studies are complicated, because of in this kind of samples is very difficult to obtain a representative sub-sample due to different proportion on grains and leaves in the same sample. Moreover, some chemical parameters such as starch, the most important nutritive parameter in maize silage for ruminant nutrition, depend on the quantity of grains in the sample.

Therefore, the first objective of the present work was the development of NIR calibrations for a range of chemical and biological parameters based on scanning undried maize silage samples.

The second step involves the calibration transfer, developed in the laboratory with a master instrument to slave. Normally, calibrations developed on master instrument can not be transferred to slave without some adjustment to the spectral data or to the calibration. The best alternative is to standardise the instruments, so that they produce identical spectra.[4] This technique has been very successful when using dried and grounded forages, but this cloning is particularly difficult for

heterogeneous and high moisture samples, such as maize silages. So, the second objective of this work was to study the possibility of transferring undried maize silage calibrations developed on one model of NIR spectrophotometer, Foss NIRSystems 5000, to Foss NIRSystems 6500, allocated in different laboratories.

Materials and methods

Development of undried maize silage calibrations

Samples

Seventy-eight (n = 78) maize silage samples were used in this study. These silages were produced in different farms across Spain during last year (2002). These samples after arriving laboratory were allowed to equilibrate to room temperature prior to scanning. After scanning sample, it is analysed using a traditional wet chemical analysis in order to quantify moisture (M), crude protein (CP), starch (ST) and organic matter enzymatic digestibility (OMED).

Chemical analyses were performed using analytical methodologies related: Moisture content was measured by oven drying sample at 60^{o}C for 24 hours. Crude protein was quantified as nitrogen (N × 6.25) using the Kjeldahl technique.[5] Starch was estimated previous gelatinisation and hydrolysis to glucose as described by Salomonson *et al*.[6] and organic matter enzymatic digestibility as described by Riveros and Argamentería.[7]

NIR analysis

Undried maize silage samples were scanned in the range 1100–2500 nm, using a scanning monochromator NIRSystems 5000 (NIRSystems, Silver Spring, MD, USA), spectra were collected as log 1/*R*. Two sub-samples of each silage were packed into the natural product cell and the spectral data were averaged. Spectral data were recorded using WINISI II ver. 1.05 software (Infrasoft International, Port Matilda, PA, USA).

NIR calibration equations were developed with 66 samples and 12 were reserved randomly for validation. Best results were obtained using modified partial least square (MPLS) regression, because this chemometric package has proven to be superior in researches using undried grass silage, internal cross validation[8] and scatter correction using standard normal variate (*SNV*) transformation.[9] Population boundaries were established with a maximum standardised H distance from the average spectrum of 3.0 to remove outliers.[10]

The mathematical treatment applied to the spectra was (2, 6, 4, 1). The first number indicate the order of derivative, the second is the gap in data points over which derivative is calculated, the third is the number of data points used in the first smoothing, and the fourth is the number of data points over which the second smooth is applied. The statistics considered more interesting to establish the boundary of calibrations were: the coefficient of determination in calibration set (*RSQ*), the standard error in calibration (*SEC*), the standard error of cross-validation (*SECV*), the coefficient of determination of cross validation (1-*VR*) and the ratio ration range/*SECV* (*RER*).[11] The ratio is a measure of the ability of a NIR model to predict a constituent.

Calibration transfer

The essential problem with all calibration transfer procedures is finding the best compensation of slave instrument against a single master. In the present work has been used the patented method

introduced by Shenk and Westerhaus[12] which separates wavelength correction from absorbance correction. A detailed description of this method is given in the ISI software manual.

It is clear that the main practical difficulty in applying the direct standardisation methods is the need of scan samples on each of the analysers involved (master and slave). Frequently a representative set of samples is selected to be scanned in the analysers. In the present work the spectra were standardised using a single undried maize silage sample spectrally close to the centre of the population. This sample was scanned firstly in the master instrument and after mailing under refrigerated conditions was scanned in the slave instrument, five hundred kilometres across Spain.

Results and discussion

Chemical data

The mean of chemical parameters and ranges of the different constituents are given in Table 1. As explained before, the most significant parameter, starch, depends directly on the grains content in the maize silage sample, show a wide variation range.

Table 1. Analytical data of maize silages under study (*n* = 78).

Parameter	Mean $_{\%DM}$	Range $_{\%DM}$
pH	3.95	3.10–5.07
Ash	4.91	3.00–9.30
Crude Protein	7.91	5.27–10.38
Starch	26.33	7.38–41.51
NDF	47.64	38.68–65.24
ADF	27.73	20.40–41.29
Crude Fibre	22.22	17.47–28.69
Fat	2.35	1.46–3.68
OMED	67.98	52.55–75.56
Metabolisable energy (MJ kg^{-1} DM)	10.35	7.68–10.61

NDF: neutral detergent fibre; ADF: acid detergent fibre; OMED: Organic matter enzymatic digestibility.

Development of calibrations

A lot of predictive models were assayed using different spectral correction techniques or spectra derivatives, but, the best results were achieved with the second derivative and *SNV* as spectra pre-treatment. The statistics of calibration obtained are given in Table 2.

Table 2. Statistical results of calibrations for moisture, crude protein, starch and organic matter enzymatic digestibility on undried maize silages.

Parameter	Mean	*SD*	*SEC*	*RSQ*	*SECV*	1-*VR*	*RER*
Moisture	67.83	4.25	0.796	0.965	1.039	0.941	19.44
Crude Protein	7.93	1.20	0.519	0.813	0.679	0.658	6.95
Starch	27.11	6.79	2.352	0.880	3.677	0.774	9.05
OMED	61.08	4.62	1.769	0.853	2.557	0.696	7.76

SD: standard deviation; *SEC*: sandard error of calibration; *RSQ*: coefficients of determination for calibration; *SECV*: standard error of cross validation; 1-*VR*: coefficients of determination for cross validation; *RER*: range/*SECV*; OMED: Organic matter enzymatic digestibility

As can be seen in Table 2 results for the studied parameters show an acceptable calibration statistics. However, it is necessary to remark that in this work the calibration has been developed with relatively few undried maize silage samples (66), and these results can be considered as preliminary results.

Calibration transfer

In general, calibration transferability is dependent upon excellent instrument standardisation. That is, to develop calibrations using spectra from the master instrument to give accurate constituent predictions for the slave instrument, the optical and the electronic characteristics of slave instrument must be matched very closely to the master instrument characteristics.

In this work the calibration transferability is not simple, as we related in material and methods, the moisture and heterogeneity of the undried maize silage make difficult to obtain the real spectral information. On another hand, master and slave instruments are allocated in different cities and undried samples could be changing quickly in a short time.

It is clear that to achieve a successful cloning of master and slave instruments the same surface of undried maize silage sample should be scanned in both analysers. In this study the single undried sample used, was wrapped in a natural product cell to be scanned in both instruments. Figure 1 shows the optical differences between master and slave spectra before and after standardisation using the ISI software 1.05. In this programme, first the method adjust for wavelengths shifts, then match the wavelength of the master and the slave instrument, being the final result a standardisation matrix. Which is stored in a file, and used to standardise the slave instrument before prediction with the master equation.

As can be seen in Figure 1 after standardisation, the slave spectra becomes closely aligned to the master spectra, across the spectral range (1100–2500 nm), showing that cloning has been successful.

These graphical results are presented statistically in Table 3, where are detailed the statistics: bias, standard error of prediction and coefficient of determination for validation. As shown in Table 3, in all cases the unstandardised spectra from the slave instrument predicted by the master equation, produced results with higher standard error of prediction than those compared to the standardised spectra. Another remarkable data showed in Table 3 is that the starch prediction values are poor (using standardised spectra *SEP*=5.453) it is due to heterogeneity of undried maize silage samples.

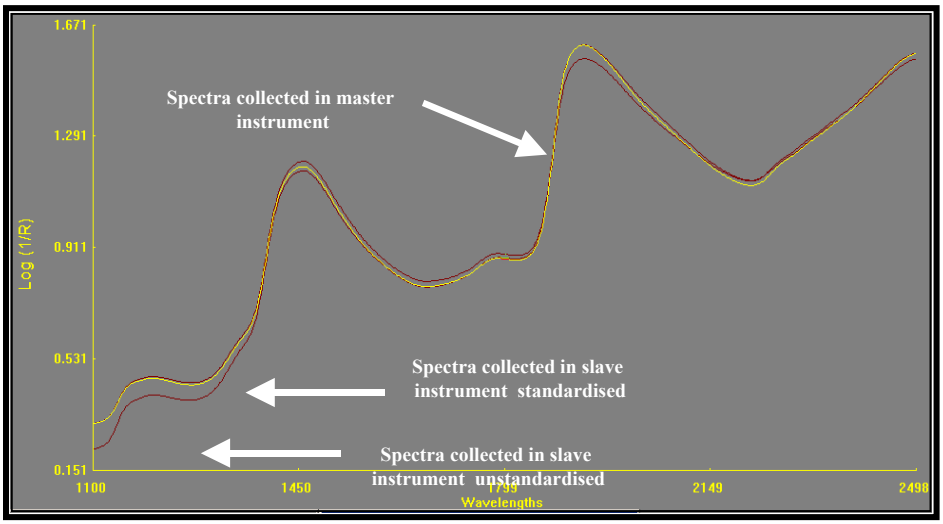

Figure 1. Comparison of the spectra of an undried maize silage samples scanned: (a) on the master, (b) on the slave and (c) slave spectra standardised to the master spectra.

Tabla 3. Statistical results for unstandardised and standardised undried maize silagepopulation.

	Unstandardised			Standardised		
Parameter	Bias	*SEP*	r^2	Bias	*SEP*	r^2
Moisture	–1.225	1.369	0.960	–0.884	1.096	0.960
Crude protein	–1.980	2.073	0.654	–1.287	1.426	0.650
Starch	12.731	13.192	0.404	–4.113	5.453	0.412
OMED	5.843	6.182	0.726	–1.138	2.368	0.710

SEP: standard error of prediction; r^2: coefficients of determination for validation

Conclusions

Despite the relatively small number of samples used in this work, we can draw two main conclusions:

1. The use of undried maize silage samples (very heterogeneous sample) to develop a NIR calibrations need a large irradiation surface area to obtain a good accuracy.

2. The use of a standardisation matrix built with only a single sample of undried maize silage scanned in master and slave showed an improvement of the statistics.

These results can be improved when the number of samples used in the NIR calibrations is increased.

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References

1. K.H. Norris, *J. Near Infrared Spectrosc.* **10,** 31 (1996).
2. . B. de la Roza, A. Martínez, S. Modroño and B. Santos, in *Near Infrared Spectroscopy: The Future Waves*, Ed by A.M.C. Davies and P. Williams. NIR Publications. Chichester, UK, p. 18 (1996).
3. R.S. Park, R.E. Agnew and R.J. Barnes, *J. Near Infrared Spectrosc.* **7,** 117 (1999).
4. J.S. Shenk, M.O. Westerhaus and W.C. Templeton, *Crop Sci.* **25,** 159 (1985).
5. A.J.M. Van Es and J. Van der Meer, in *Methods of Analysis for Predicting the Energy and Protein Value of Feeds for Farm Animals.* Institute for Livestock Feeding and Nutrition Research, Lelystad, The Netherlands (1980).
6. A.C. Salomonsson, O. Theander and E. Westerlund, *Swedish J. Agric. Res.* **14,** 111 (1984).
7. E. Riveros, A. Argamenteris, *Avances en Producción Animal* **12,** 49 (1987).
8. J.S. Shenk and M.O. Westerhaus, *Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy*. Infrasoft International, ISI, Port Matilda, USA (1993).
9. R.J. Barners, M.S. Dhanoa and S.J. Lister, *Appl. Spectrosc.* **43,** 772 (1989).
10. J.S. Shenk and M.O. Westerhaus, *Crop Sci.* **31,** 1148 (1991).
11. P. Williams and D. Sobering, in *Near Infrared Spectroscopy: The Future Waves*, Ed by A.M.C. Davies and P. Williams. NIR Publications. Chichester, UK, p. 18 (1996).
12. J.S. Shenk and M.O. Westerhaus, US Patent No. 4,866,644 (12 September 1989).