Determination of inorganic elements in animal feeds by NIRS technology and a fibre-optic probe

Inmaculada González-Martín *, Noelia Álvarez-García,
Claudio González-Pérez, Virginia Villaescusa-García

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de CC, Químicas, C/Plaza de la Merced s/n, 37008 Salamanca, España

Abstract

In the present work we study the use of near infra-red spectroscopy (NIRS) technology together with a remote reflectance fibre-optic probe for the analysis of the mineral composition of animal feeds. The method allows immediate control of the feeds without prior sample treatment or destruction through direct application of the fibre-optic probe on the sample.

The regression method employed was modified partial least squares (MPLS). The calibration results obtained using forty samples of animal feeds allowed the determination of Fe, Mn, Ca, Na, K, P, Zn and Cu, with a standard error of prediction (SEP(C)) and a correlation coefficient (RSQ) of 0.129 and 0.859 for Fe; 0.175 and 0.816 for Mn; 5.470 and 0.927 for Ca; 2.717 and 0.862 for Na; 4.397 and 0.891 for K; 2.226 and 0.881 for P; 0.153 and 0.764 for Zn, and 0.095 and 0.918 for Cu, respectively.

The robustness of the method was checked by applying it to 10 animal feeds samples of unknown mineral composition in the external validation.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Animal feed; Inorganic element; Determination; NIRS; Fibre-optic probe

1. Introduction

The composition of animal feeds includes added mineral and vitamins, which are essential for the functioning of living beings, although the amounts supplied to animals must be present in the correct proportions since deficiencies in these essential components lead to diseases such as anaemia, delays in growth, etc., while their massive consumption produces intoxication and the accumulation of such compounds in the organism. It is therefore crucial to control the safety levels of their concentrations and tolerance to them.

Minerals in agricultural products probably exist in both inorganic and organic complexes. The concentration of inorganic components in forage crops varies according to crop maturity, plant parts, temperature, amount of fertilisers applied to the crop, soil fertility and soil physical characteristics [1]. Reference methods for mineral analysis include inductively coupled argon plasma (ICP), atomic absorption spectroscopy (AAS) and X-ray

fluorescence spectroscopy (XRF) [2]. Because NIRS measures absorption by molecular bonds, its use for pure minerals may seems to have no sense. However, prediction of some minerals in forages by NIRS may be possible through their association with the organic matrix [3,4]. Only a few reports were found related to the use of NIRS for macro and trace minerals in both grasses and hay samples [5–7], in natural grasses [8] and in botanical fractions of semi-arid grasslands [9] or legumes [10]. In our search of the literature, we failed to find references to the determination of mineral substances in feed by NIRS with a fibre-optic probe. Thus, the aim of the present work was to explore the potential and accuracy of NIRS for predictions of Fe, Mn, Ca, Na, K, P, Zn and Cu in animal feeds without prior sample treatment, using direct application of the probe on the feed.

2. Experimental

2.1. Samples

Forty samples of feeds with different mineral compositions and physical aspect were used. The samples corresponding to

---

* Corresponding author. Tel.: +34 23 29448; fax: +34 23 29448.
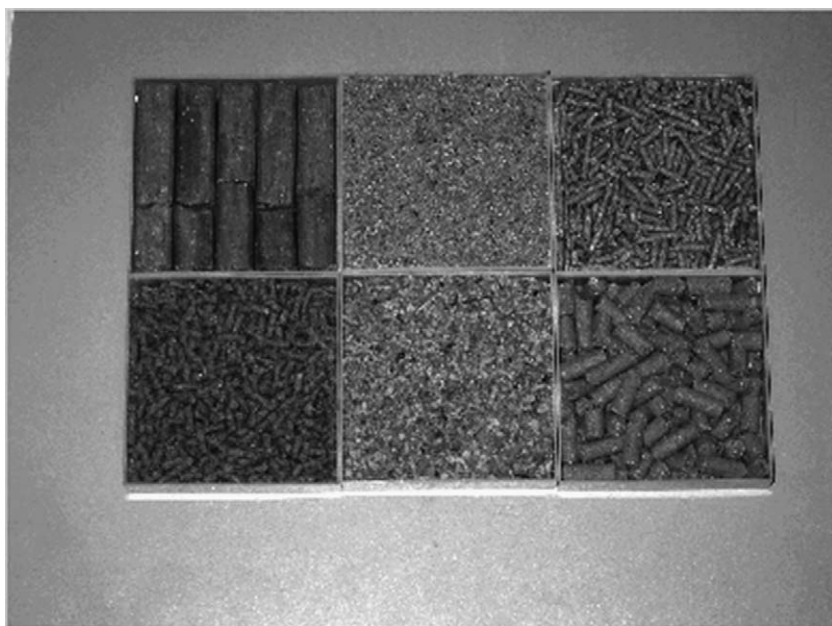*E-mail address:* inmaglez@usal.es (I. González-Martín).

Fig. 1. Types of texture and presentation of the feed samples used.

the stages of weaning, growth, maintenance, lactation and fattening, and with different textures: blocks or tablets (5 samples), granules of different sizes (29 samples) and meal (6 samples). Fig. 1 shows the different textures and forms of presentation of the feeds used in the work.

2.2. Reference chemical analyses

To determine the chemical composition of the reference samples, the feeds were ground, homogenised and desiccated in an oven at 80–90 °C for 24 h. Once dry, the feeds were mineralised in a microwave system, measuring the mineral composition in the resulting solution by ICP.

Microwave digestion was accomplished in a Milestone MLD-1200 oven. One gram of dried ground sample was placed in a Milestone HPV 80 capsule and subjected to two steps: in the first, 5 mL of HNO_3 (conc.) was added, applying a power of 300 W for 5 min, while in the second step the sample was allowed to cool, after which a further 5 mL of HNO_3 (conc.) and 1 mL of H_2O_2 (30%) were added, applying a power of 300 W for 7 min. Once the samples had cooled to room temperature, volume was brought up to 100 mL with distilled water and the samples were stored at 4 °C until analysis. The process was carried out in duplicate for each sample.

Measurements were made using ICP on a Perkin-Elmer Plasma II optic ICP device. The results obtained—the means of two determinations—are expressed as ppm in solution obtained after the mineralization step (1 g of dry sample/100 mL), corresponding to mg/kg of feed.

2.3. NIR spectroscopy

A Foss NIRsystems 5000, with a standard 1.5 m 210/7210 bundle fibre-optic probe, Ref no. R6539-A, was used. The probe

employs a remote reflectance system and uses a ceramic plate as reference. The window is of quartz, with a 5 cm × 5 cm surface area, measuring reflectance in the IR zone close to 1100–2000 nm (Fig. 2).

Measurements were carried out in reflectance mode between 1100 and 2000 nm. Spectra were recorded at intervals of 2 nm, performing 32 scans for both the reference and samples. The average spectrum was used for NIR analysis. The software used was Win ISI 1.05, installed on a Hewlett-Packard Pentium III computer.

2.4. Statistical analyses

The Mahalanobis distance (*H* statistic) is calculated from principal component analysis scores. The results indicate how different a sample spectrum is from the average sample of the set [11]. A sample with an *H* statistic of ≥3.0 standardised units
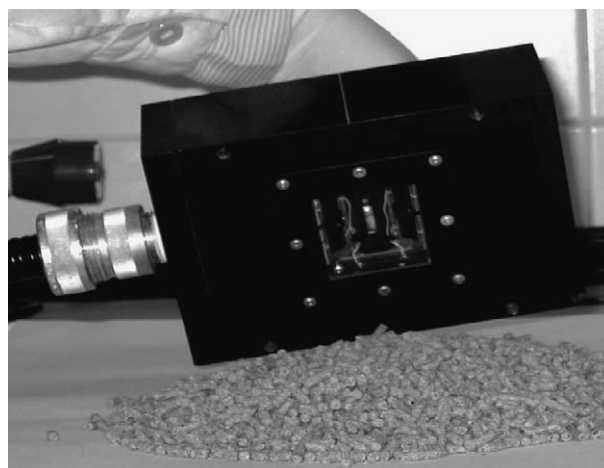

Fig. 2. Recording of the spectra of feed samples with the probe.

from the mean spectrum is defined as a global *H* outlier and is then eliminated from the calibration set.

Calibrations were performed by modified partial least squares regression (MPLS). To optimise the accuracy of calibration, several scattering corrections and mathematical treatments were tested (standard normal variate, SNV; De-trending, DT; multiplicative scatter corrections, MSC; first derivative and second derivative). The best one was selected for each constituent on the basis of the highest RSQ (multiple correlation coefficient) and the lowest standard error of calibration and cross-validation (SEC and SECV, respectively). Assessment of the calibration model was performed by cross-validation. In this method, the set of calibration samples is divided in groups, using one of them to check the results (prediction) and the remaining to construct the calibration model. The model is repeated, as many times as there are groups in such a way that all pass through the calibration set and the prediction set.

Samples from the validation set were then analysed with these equations, which gave a standard error of prediction corrected (SEPC) and bias (mean of residuals, defined as the difference between the laboratory value and the value predicted by the equation) for each constituent. In this step, samples with high residual values were eliminated, using the $T > 2.5$ criterion.

After the calibration equations had been obtained, they were subjected to external validation by application to a set not involved in the calibration process (10 samples) checking the functioning of the fibre-optic probe. Spectra were recorded in triplicate and the spectral average was taken. The calibration equations obtained in the development of the procedure were applied and the predicted values were compared with the laboratory results obtained later.

3. Results and discussion

3.1. Chemical analyses and spectral information

The results corresponding to the chemical composition of the inorganic elements in 40 samples of feed are shown in Table 1 as the means of two determinations and are expressed as ppm in solution (1 g of dry sample in 100 mL), corresponding to mg/kg of feed. Of interest in this table are the elevated standard deviations for Ca and K, followed in order by those of Na and P, owing to the variability in the composition of the samples selected.

Once the number of principal components had been determined, anomalous spectra were detected using the Mahalanobis

Table 1
Statistical overview of chemical analyses (all units in ppm)

Components	Minimum	Maximum	Mean	S.D.
Fe	1.56	4.10	2.43	0.56
Mn	0.54	2.22	1.05	0.52
Ca	75.20	187.12	116.23	24.9
Na	15.72	50.74	33.78	8.56
K	67.86	139.30	97.65	14.3
P	37.44	64.82	51.04	7.21
Zn	0.12	1.32	0.38	0.35
Cu	0.56	1.75	1.17	0.34

distance (*H* statistic), establishing $H = 3$ as the limit value. Thus, those spectra whose *H* distance was greater than 3.0 were considered different from the spectral population and were discarded. The principal components obtained lie between 6 and 8, depending on the inorganic element analysed, in all cases accounting to 95% of the spectral variability (Table 2).

Fig. 3 shows the spectra of a sample of feed together with the treatments that proved to be optimum for the calibration of Fe, Zn, Mn and P elements. The best results were obtained by applying mathematical treatments such as the multiplicative scatter correction (MSC) method, a technique initially used by Geladi et al. [12] that prevents the effects of dispersion in ground samples from becoming superimposed over the chemical signals and the SNV-DT and 1st derivative spectral treatment. Those transformations have been introduced to correct problems appearing in the log(1/*R*) form associated with sample particle size [13]. The SNV-DT were introduced not only for reduction of multicollinearity but also to calculate spectral differences by reducing the confounding effects of baseline shift and curvature; without treatment, indicated in the table with "none".

Once the number of principal components had been calculated, and prior to obtaining the calibration algorithm, the samples that for spectral (*H* criterion) or chemical (*T* criterion) reasons were deficient in the calibration set have been removed from the model. The number of samples remaining that have been used in the calibration algorithm are indicated in Table 3.

3.2. Determination of inorganic components in feeds

3.2.1. Calibration equations

Calculation of the statistical parameters of the calibration equations for each component is shown in Table 3, together

Table 2
Principal component analysis: mineralogical composition in feeds

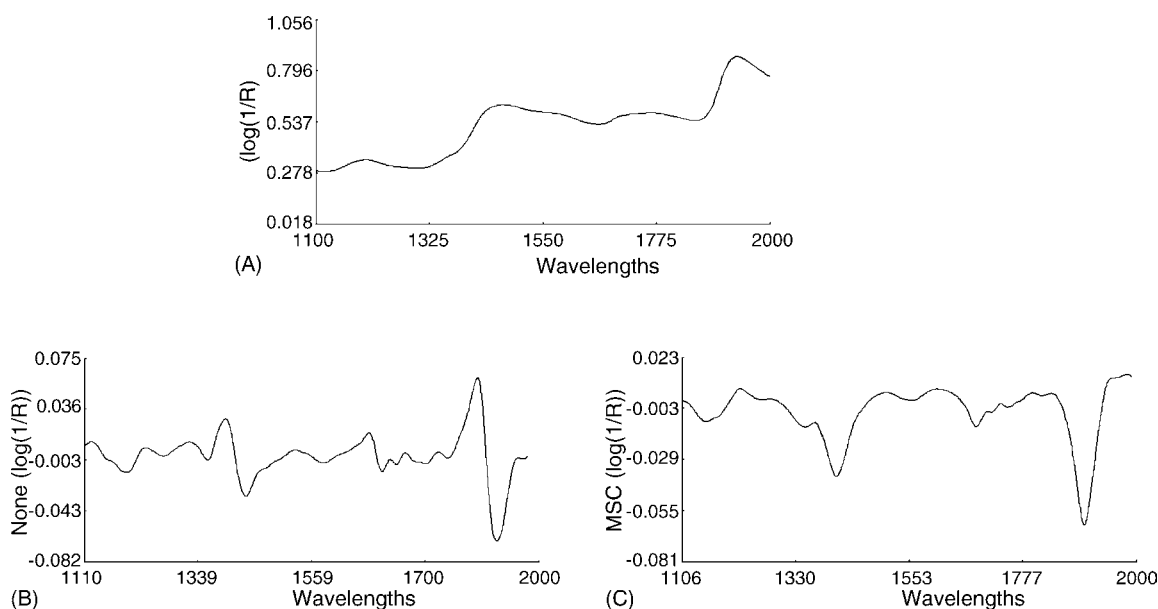| Components	Mathematical treatment	Number of principal elements	Probability explained (%)
Fe	None/2nd derivative	8	95.58
Mn	Standard MSC/1st derivative	7	98.30
Ca	SNV-DT/1st derivative	7	98.22
Na	None/1st derivative	6	98.97
K	SNV/2nd derivative	8	98.20
P	Standard MSC/1st derivative	7	98.30
Zn	None/2nd derivative	8	98.35
Cu	DT/none	6	99.65

Fig. 3. Raw spectrum and results of treating it mathematically. (A) NIR spectrum, (B) Fe and Zn: spectrum corrected using the none/2nd derivative and (C) Mn and P: spectrum corrected using the MSC/1st derivative.

with the best treatments corresponding to the MPLS calibration of each inorganic element in the feeds.

Scrutiny of the statistical descriptors of calibration reveals that the RSQ parameters are very good for each of the inorganic elements, although in the case of Ca and K, and to a lesser extent of Na and P, the SEC and SECV errors are high, but always lower than the standard deviations of the reference chemical data.

3.2.2. Validation

3.2.2.1. Internal validation (prediction).
Internal evaluation of the model was performed by cross-validation using seven cross-validation sets in all cases, employing one of them to check the results (prediction) and the other six to construct the calibration model. The model was implemented as many times as there were groups, such that all of them passed through the calibration set and the prediction set.

The results of the statistical descriptors of the internal validation in NIR for the inorganic elements analysed in the feed samples are shown in Table 4. From the SEP, SEP(C) and bias results, it may be inferred that the calibration model for Fe, Zn, Mn and Cu is robust and that it allows the determination of these elements with excellent results; the results are slightly less sat-

isfactory for Na and P and are not so acceptable for Ca and K, as shown in the statistical descriptors, SEC and SECV.

3.2.2.2. External validation.
Once the calibration equations for the determination of the inorganic composition of the feed samples had been obtained, we proceeded to the external validation step, which consisted of applying the equations to a set of 10 samples that did not belong to the calibration set. The procedure implemented was as follows. The spectra were recorded in triplicate and the spectral mean was taken. The calibration equations obtained in the development of the work were applied and the predicted values were compared with the laboratory results, which were obtained later by digesting 0.5 and 1 g of feed sample in the microwave device. Table 5 shows the differences found between the reference method (ICP) and the NIRS technique. It may be seen that there are differences between the values of the residuals and the error in percentage, depending on the amount digested (microwave), which confirms that compositional variability is due to the heterogeneity of the samples and their texture.

Table 3
Statistical descriptors of calibration: inorganic components in feeds

Components	N	Mathematical treatment	RSQ	SEC	SECV
Fe	40	None/1st derivative	0.859	0.145	0.279
Mn	36	Standard MSC/2nd derivative	0.816	0.196	0.357
Ca	36	DT/2nd derivative	0.927	6.115	15.89
Na	38	Standard MSC/2nd derivative	0.862	2.966	5.690
K	39	Standard MSC/2nd derivative	0.891	4.774	7.827
P	37	DT/2nd derivative	0.881	2.469	6.164
Zn	40	None/3rd derivative	0.764	0.162	0.211
Cu	38	DT/2nd derivative	0.918	0.103	0.168

Table 4
Statistical descriptors of validation

Components	RSQ	SEP	SEP(C)
Fe	0.888	0.127	0.129
Mn	0.853	0.172	0.175
Ca	0.942	5.381	5.470
Na	0.884	2.674	2.717
K	0.907	4.332	4.397
P	0.903	2.192	2.226
Zn	0.789	0.150	0.153
Cu	0.932	0.093	0.095

Determination of inorganic components in feeds (slope = 1.000 and bias = 0.000).

Table 5
External validation

Compounds	Amount of sample used in the digestion (g)	Mean of residuals	Differences (%)
Fe	1	0.13	5.3
	0.5	0.16	6.4
Mn	1	−0.05	5.5
	0.5	0.08	7.9
Ca	1	6.23	5.0
	0.5	7.16	5.7
Na	1	−2.83	7.3
	0.5	−3.15	8.3
K	1	−11.8	12.2
	0.5	−9.59	9.8
P	1	−4.49	9.2
	0.5	−2.76	5.5
Zn	1	0.35	23.8
	0.5	0.29	20.6
Cu	1	0.09	–
	0.5	0.06	–

Differences between the reference method (ICP) and the NIRS technique. Precision of the ICP reference concentrations expressed in ppm: Fe, ±0.04; Mn, ±0.06; Ca, ±0.7; Na, ±2; K, ±1.5; P, ±1; Zn, ±0.1; Cu, ±0.09.

The highest residuals were observed for Ca, K and P, although the error was not above 10%. In the case of Cu, the % error was not calculated because the precision of the ICP reference concentrations expressed in ppm (0.09) included the Cu contents in the feeds. It is interesting with NIR, however, that is was possible to detect those levels. The determination of Ca, Na and K by ICP indicated a very high standard deviation. Upon obtaining the calibration and cross-validation errors by NIRS, it was found that the errors were considerably reduced, especially for Ca and K.

NIR responds to rotational and vibrational energies of the hydrogen bond; thus, inorganic salts will be transparent in the NIR region. The fact that it is possible to predict the concentration of mineral elements by NIR is due to the association of organic or hydrated inorganic molecules present in the plant tissues of the raw material. This would explain the high standard deviation obtained for K and Ca, which could be due to the inverse relationship between the water retaining capacity and the content in K and Ca. Na exerts the opposite effect to K in water retention in tissues, this being seen in the standard

deviations of the feeds. These antagonistic effects were highlighted by Swift and Berman [14] and also reported in the work of González-Martín et al. [15] in meat samples, where the mineral composition of K and Ca shows considerable variability. Regarding P, the low electronegativity of this element gives rise to the formation of very weak hydrogen bridges. Despite this, good results are obtained, although it is necessary to use the spectrum third derivative in the mathematical treatment.

Thus, the calibration equations obtained are of sufficient quality to know with predicting the mineral composition of different feed types using NIRS and a fibre-optic probe. The results show that the analytical method discussed, employing a fibre-optic probe in direct contact with the sample, is rapid, non-contaminant and does not involve previous sample treatment or manipulation.

References

[1] A. MacPherson, in: D.I. Givens, E. Owen, R.F.E. Axford, H.M. Omedi (Eds.), Forage Evaluation in Ruminant Nutrition, CABI Publishing, Wallingford, UK, 2000, pp. 345–373.
[2] J.S. Shenk, J. Workman, M.O. Westerhaus, in: D.A. Burns, E.W. Ciurczak (Eds.), Handbook of Near Infrared Analysis, Marcel Dekker, USA, 1992, pp. 383–431.
[3] D.I. Givens, E.R. Deaville, J. Agric. Res. 50 (1999) 1131.
[4] J.S. Shenk, M.O. Westerhaus, M.R. Hoover, J. Dairy Sci. 62 (1979) 807.
[5] D.H. Clark, H.F. Mayland, R.C. Lamb, Agron. J. 79 (1987) 485.
[6] S. Saiga, T. Sasaki, K. Nonaka, K. Takahashi, J. Jpn. Grass Soc. 35 (1989) 228.
[7] K.F. Smith, S.E. Willis, P.C. Flinn, Aus. J. Agric. Res. 42 (1991) 1399.
[8] B.R. Vázquez de Aldana, B. García Criado, A. García Ciudad, M.E. Pérez Corona, Commun. Soil Sci. Plant Anal. 26 (1995) 1383.
[9] A. Ruano-Ramos, A. García Ciudad, B. García Criado, Anim. Feed Sci. Technol. 77 (1999) 331.
[10] D. Cozzolino, A. Moron, Anim. Feed Sci. Technol. 11 (2004) 161.
[11] P.C. Williams, K. Norris, Near Infrared Technology in the Agricultura and Food Industries, American Association of Cereal Chemists Inc., St. Paul, Minnesota, U.S.A., 1997.
[12] P. Geladi, D. Mac Dougall, H. Martens, Appl. Spectrosc. 39 (3) (1985) 491.
[13] M.S. Dhanoa, S.J. Lister, R.J. Barnes, Appl. Spectrosc. 49 (6) (1995) 765.
[14] C.E. Swift, Berman, Food Technol. 13 (1959) 365.
[15] I. González-Martín, C. González-Pérez, J. Hernández-Méndez, N. Álvarez-García, Anal. Chim. Acta 468 (2002) 293.