Available online at www.sciencedirect.com

SCIENCE DIRECT®

Talanta 69 (2006) 706–710

Talanta

www.elsevier.com/locate/talanta

Near-infrared spectroscopy (NIRS) with a fibre-optic probe for the prediction of the amino acid composition in animal feeds

Inmaculada González-Martín*, Noelia Álvarez-García,
José Miguel González-Cabrera

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

Received 16 May 2005; received in revised form 26 October 2005; accepted 4 November 2005
Available online 15 December 2005

Abstract

The amino acids alanine, aspartic acid, glutamic acid, glycine, phenylalanine, valine, lysine, proline, and tyrosine present in feeds with different textures (blocks, tablets, granules and flour (meal) and used in different stages of animal feeding regimes (lactation, growth, maintenance, etc.) were analysed using near-infrared reflectance spectroscopy (NIRS) technology together with a remote reflectance fibre-optic probe. The method allows immediate control of the animal feeds without prior sample treatment or destruction through direct application of the fibre-optic probe on the sample.

The regression method used was Modified Partial Least Squares (MPLS). The equations developed to determine the amino acid contents of the feeds afforded high values for the RSQ coefficient (0.814–0.963) in all the amino acids with the exception of lysine (0.687). The statistical prediction descriptors SEP, SEP(C) (with values between 0.134 for valine and 0.015 for aspartic acid) and bias indicated that the amino acid values in feeds predicted with NIRS with a fibre optic probe are comparable to those obtained with the chemical ion-exchange HPLC method.

Keywords: Animal feed; Amino acids; NIRS calibration; Fibre-optic probe

---

1. Introduction

Amino acids are important components in the composition of animal feeds and knowledge of their levels and control of these allows the nutritional value of feeds to be evaluated and enables determination of the correct amounts of them to be added to feeds [1]. However, the chemical determination of amino acids is a long and tedious process, involving the analysis of hydrolysed protein with an amino acid analyser, which is accurate but also operationally complex, time-consuming, and expensive.

Since 1978, when Rubenthaler and Bruinsma [2] calibrated the content of lysine in wheat and barley, the development of the near-infrared reflectance spectroscopy (NIRS) technique combined with chemometric calibration algorithms has allowed amino acids to be measured in feed ingredients Williams [3], Workman [4], Van Kempen and Bodin [5], Dyer and Feng [6]. Recently, Fontaine et al. [7–9] has published the results obtained in the calibration of amino acids in feeds rich in protein carried out at the Degussa laboratory, those obtained in different cereals, and the results of a collaborative study that allowed standardisation in 44 NIRS devices. In all works consulted, the samples used were finely ground and conventional measuring cells were employed.

The aim of the present work was to perform NIRS calibration with a fibre optic probe that would allow us to predict the composition of amino acids in feeds, with no treatment of manipulation of the samples, by direct application of the probe to the feeds.

2. Materials and methods

2.1. Samples

Fifty samples of feeds with considerable variability in their composition and physical aspect – depending on the target

---

* Corresponding author. Tel.: +34 923 294483; fax: +34 923 294483.
E-mail address: inmaglez@usal.es (I. González-Martín).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.11.015

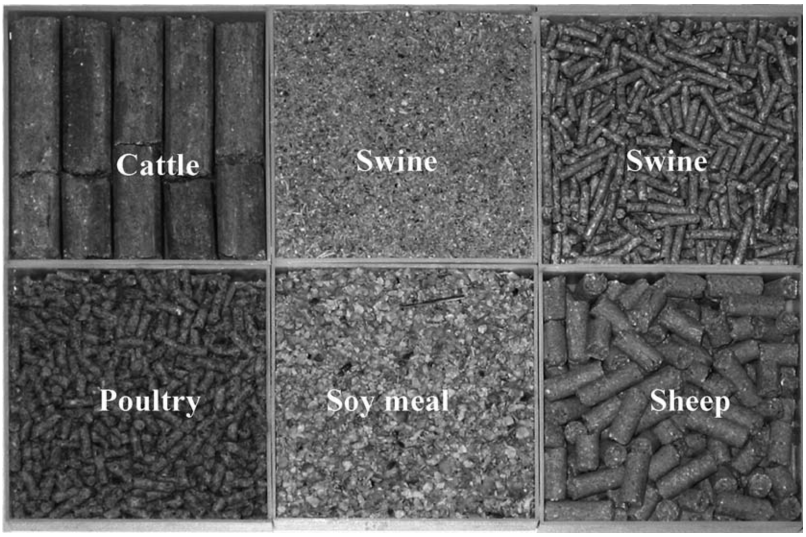

Fig. 1. Different textures of the feeds analysed.

species and the age of the animal – were used. Specifically, there were 18 samples destined for cattle; 12 for sheep; 10 for swine; 5 for poultry; 3 for horses and 2 for rabbits, corresponding to the stages of weaning, growth, maintenance, lactation and fattening, and with different textures: blocks or tablets, granules of different sizes and meal. Fig. 1 shows some of the feeds used.

2.2. 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, amino acids were analysed with the Official Method 994.12 of the AOAC International of amino acids analysis in feed, by hydrolysis in HCl medium and HPLC determination.

An amount of sample (between 0.3 and 0.5 g) was weighed so that the content in nitrogen would be close to 10 mg; 5 mL of performic acid was added, and the samples were left in an ice bath for 16 h. Following this, the performic acid was destroyed with sodium metabisulphate; 50 mL of 6 M HCl was added, and the mixture was left for 14 h at 11 °C for protein hydrolysis to be completed. After this time, the samples were left to cool, were filtered, and pH was adjusted to 2.20 with NaOH. The different amino acids present in the samples were determined by ion exchange chromatography.

2.3. NIRS spectroscopy

A Foss NIRsystems 5000 with a standard 1.5 m 210/210 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.

Measurements were carried out in reflectance mode between 1100 and 2000 nm. The spectra were recorded at intervals of 2 nm, performing 32 scans for both the reference and samples. The average spectrum of three recordings 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, Williams and Norris [10]. A sample with an H statistic of ≥ 3.0 standardised units 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 SEV, 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 ones 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.

3. Results and discussion

3.1. Chemical analyses and spectral information

The chemical compositions of the 50 feed samples used for calibration are shown in Table 1 as means of two determinations and expressed in % weight in the feeds. The compositions and standard deviations are typical of this type of sample. The contents of the different amino acids revealed a broad range of variability, which is important when searching for calibration equations to be used later in the prediction. Seven of the amino acids – alanine, aspartic acid, glutamic acid, glycine, phenylalanine, and valine – were present in all the samples, while lysine, proline and tyrosine were present in most of them.

Table 1
Statistical overview of chemical analysis (all units in %), $N = 50$

Amino acid	Minimum	Maximum	Mean	S.D.
Alanine	0.043	0.793	0.299	0.161
Aspartic acid	0.036	0.362	0.120	0.065
Glutamic acid	0.376	2.913	1.381	0.742
Glycine	0.009	0.652	0.160	0.152
Lysine	0.041	0.752	0.311	0.187
Phenylalanine	0.147	1.572	0.543	0.271
Proline	0.057	0.349	0.177	0.076
Tyrosine	0.038	0.951	0.175	0.177
Valine	0.016	1.847	0.423	0.427

Table 2
Principal component analysis: amino acids in feeds

Amino acid	Mathematical treatment	No. of principal components	Probability explained (%)
Alanine	DT/2nd derivative	13	99.33
Aspartic acid	Standard MSC/2nd derivative	11	99.21
Glutamic acid	DT/2nd derivative	13	99.33
Glycine	SNV/2nd derivative	11	99.38
Lysine	None/2nd derivative	8	99.02
Phenylalanine	Standard MSC/2nd derivative	13	99.51
Proline	Standard MSC/3rd derivative	11	99.22
Tyrosine	None/1st derivative	9	99.57
Valine	DT/2nd derivative	8	99.00

Table 3
Statistical calibration descriptors for the NIR determination of amino acids in animal feed

Amino acid	Mathematical treatment	RSQ	SEC	SECV
Alanine	Standard MSC/2nd derivative	0.963	0.026	0.151
Aspartic acid	SNV/2nd derivative	0.894	0.016	0.066
Glutamic acid	SNV/2nd derivative	0.847	0.060	0.155
Glycine	Standard MSC/2nd derivative	0.852	0.036	0.101
Lysine	None/2nd derivative	0.687	0.116	0.142
Phenylalanine	Standard MSC/2nd derivative	0.959	0.145	0.746
Proline	SNV/2nd derivative	0.877	0.027	0.102
Tyrosine	SNV/2nd derivative	0.941	0.023	0.082
Valine	SNV/2nd derivative	0.814	0.144	0.340

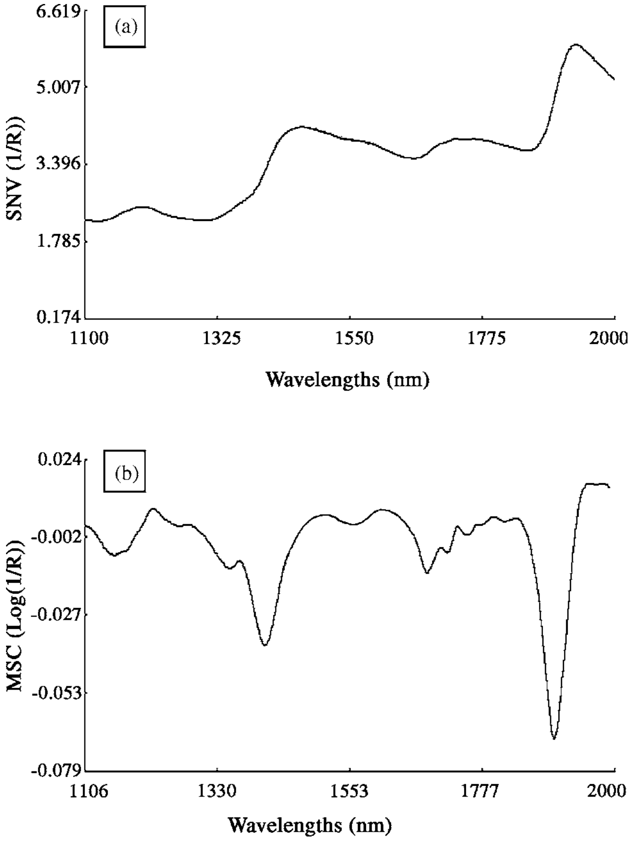

Fig. 2. (a) Spectrum of a feed sample obtained by application of the probe. (b) Spectrum corrected mathematically (Standard MSC/2nd derivative) for alanine.

Fig. 2 shows the spectrum obtained by direct application of the probe to a feed sample, together with the spectrum generated with the best mathematical treatment for alanine (Standard MSC/2nd derivative). The overall set of treatments obtained to calculate the principal components, the number of principal components, and the variability explained is shown in Table 2. In all cases, between 8 and 13 principal components were selected, accounting for a spectral variability above 99%.

To detect anomalous spectra, the Mahalanobis distance (H) was used, setting $H = 3$ as the limit value. With this criterion, one spectrum was eliminated in the case of lysine and another one in the case of valine.

Table 4
Applicability (%) of the calibration equations of amino acids in feeds

Amino acid	Est. Min.	Est. Max	Mean	S.D.
Alanine	0.000	0.699	0.284	0.138
Aspartic acid	0.000	0.260	0.111	0.050
Glutamic acid	0.000	0.739	0.277	0.154
Glycine	0.000	0.404	0.125	0.093
Lysine	0.000	1.141	0.518	0.208
Phenilalanine	0.000	3.473	1.329	0.715
Proline	0.000	0.405	0.177	0.076
Tyrosine	0.000	0.437	0.147	0.097
Valine	0.000	1.354	0.349	0.335

3.2. Determination of amino acids in feeds. Calibration equations

The statistical calibration parameters for each amino acid are shown in Table 3. The equations developed for the determination of the amino acid content in feeds have high values for the RSQ coefficient (0.814–0.963) in all amino acids except lysine (0.687). The standard errors of calibration (SEC) and of cross-validation (SECV) are acceptable.

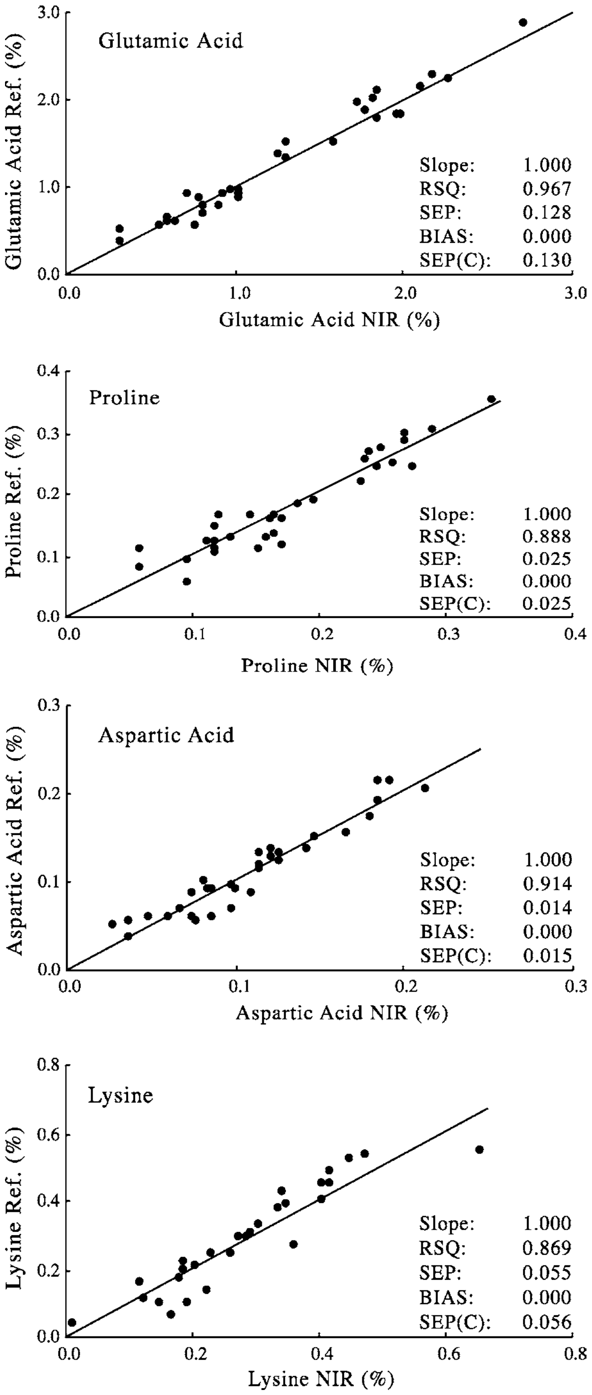

Fig. 3. Comparison of the reference values with those predicted by the calibration equations (statistical prediction descriptors).

3.3. Internal validation (prediction)

Model evaluation was performed by cross-validation. In this method, the set of calibration samples is divided into a series of subsets: in the present case, 7. Of these, 6 were taken for the calibration set and one for the prediction set; the process was repeated as many times as there were sets, such that all passed through the calibration set and the prediction set. Using this process, we validated the model used and checked its prediction capacity.

The predicted values gave validation errors that were combined into SEP(C). In this step, samples with high residual values were removed using the $T > 2.5$ criterion. Thus, one sample was removed in the case of alanine and two in the case of aspartic acid. Fig. 3 shows the correlation of the values obtained in the laboratory with respect to those predicted by NIR with the fibre optic probe for the amino acids in feeds and the statistical descriptors of prediction. From the SEP, SEP(C) and BIAS results, it may be inferred that the calibration model for alanine, aspartic acid, glutamic acid, glycine, phenylalanine, valine, lysine, proline and tyrosine is robust and that it allows the determination of these amino acids with excellent results.

The concentration ranges and standard deviations for each amino acid in which it is possible to apply the calibration equations obtained with direct measurement of the NIR spectra with the probe and HPLC as the reference method are shown in Table 4. The results obtained reveal that the values of amino acids in feeds predicted by NIRS are comparable to those obtained with the chemical method employing ion-exchange HPLC.

4. Conclusions

The determination and prediction of the amino acids alanine, aspartic acid, glutamic acid, glycine, phenylalanine, valine, lysine, proline and tyrosine were performed in feed samples by near-infrared reflectance spectroscopy with a remote reflectance fibre optic probe. The results obtained show that the analytical technique employing direct application of the fibre optic probe to the samples is rapid, does not pollute, and does not require prior treatment of manipulations of the samples. A further advantage is that it can be performed regardless of the presentation of the feeds (tablets, granules, grain, meal) and of the age of the animals (lactation, growth, maintenance, etc.) that are to receive the feed.

More research is required to confirm these results so that the method can serve as a quality control of the content of amino acids in feed manufacturing processes.

References

[1] K. Baker, in: C. Ammerman, D.H. Baker, A.J. Lewis (Eds.), Bioavailability of Nutrients for Animals Amino Acids, Minerals and Vitamins, Academic Press, New York, 1995.
[2] G.L. Rubenthaler, B.L. Bruinsma, Crop Sci. 18 (1978) 1039.
[3] P.C. Williams, K.R. Preston, K.H. Norris, P.M. Starkey, J. Food Sci. 49 (1984) 17.
[4] J. Workman, in: Proceedings of the Georgia Nutrition Conference for the Feed Industry, University of Georgia, Athens, GA, 1991, p. 37.

[5] T. Van Kempen, J.C. Bodin, Anim. Feed Sci. Technol. 76 (1998) 139.

[6] D.J. Dyer, P. Feng, Near infrared spectroscopy, in: Proceedings of the 7th International Conference, NIR Publications, Chichester, UK, 1996, p. 490.

[7] J. Fontaine, J. Hörr, B.J. Schirmer, Agric. Food Chem. 49 (2001) 57.

[8] J. Fontaine, B.J. Schirmer, J. Hörr, J. Agric. Food Chem. 50 (2002) 3902.

[9] J. Fontaine, J. Hörr, B.J. Schirmer, J. Agric. Food Chem. 52 (2004) 701.

[10] P.C. Williams, K. Norris, Near Infrared Technology in the Agricultural and Food Industries, American Association of Cereal Chemists Inc. St. Paul, Minnesota, U.S.A., 1987.