Calibration strategies for prediction of amino acid content of poultry feeds

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Introduction

The characterization of poultry feeds requires the knowledge of their composition in terms of the nutritionally important chemical compounds, as crude protein, fat, starch and amino acids (AAs). Essential AAs such as lysine and the sulphur containing AAs (methionine and cysteine) must reach sufficient levels to allow optimal performance of animals. The chemical determination of individual AAs by classical methods using high pressure liquid chromatography procedures [1] is time consuming and expensive which makes them unsuited for routine use, particularly in developing countries.

This study investigated the potential of near infrared (NIR) spectroscopy to estimate the amino acid content of a range of poultry feeds from the east African countries of Kenya, Tanzania, Uganda, Ethiopia, Sudan, Eritrea and Burundi as an indicator of their nutritive value. The aim of this study was to provide a reliable overview of the nutritive value of poultry feeds available on the local markets in order to advise farmers on feed use and to help feed millers improve the quality of their products [2].

AAs, characterised by their amino functional group $-NH_2$), are the basic components of proteins, and their level is therefore linked to crude protein level. This is an "advantage" for NIR spectroscopy calibration of AAs in raw materials [3], since the strong correlation between AA and protein content helps the NIR calibration to predict nutritive value. This explains the relative success of this technique in raw materials [4]. Conversely, complete mixed feeds may contain variable amounts of different materials, each containing different amounts of individual AAs. The correlation between individual AA and protein content is therefore often weaker when applied to complete feeds than to raw materials. Also it is a common practice in feed formulation to add purified AAs to the diet to improve nutritional value [1], which can further distort the relationship. Therefore attempts to build calibrations for the AA content of poultry feeds often fail to provide robust models. This study aimed at evaluating the performance of calibration databases built with samples experimentally enriched with purified AAs.

Materials and methods

In the present experiment, 130 poultry feed samples (Set 1) from various East African countries were analysed for lysine, methionine and cysteine levels by standard reference high performance liquid chromatography (HPLC) methods [5]. An additional set of 110 samples (Set 2) was obtained by adding of pure lysine, methionine and cysteine to some of the original feeds. Each sample could be enriched with one or several AAs. This was essential since otherwise NIR spectroscopy could have detected a global amino nitrogen level effect instead of the real level of each individual AA. The levels of AA added ranged from 0 to 8 $g.kg^{-1}$ for lysine and 0 to 10 $g.kg^{-1}$ for methione and cysteine, which kept the total AA content reasonably high. This was never more than twice the "natural" concentration met in common feeds. Forty of the enriched samples were re-analysed by

the reference HPLC method [5] to check the quality of the mixing procedure and the absence of any analytical bias.

The 240 samples, comprising Set 1 and Set 2, were re-ground through a 0.5 mm sieve, after adding the supplemental AA in order to homogenise the mixture. NIR spectra were collected in duplicate (2 different cup fillings) in reflectance mode using a Foss 6500 spin cell instrument (Foss NIRSystems, Silver Spring, MD, USA) and averaged. Calibration equations were built after mathematical pre-processing of the data calculating the standard normal variate and detrending on the second derivative of the spectra. Visible wavelengths (400 – 800 nm) were discarded because they introduced instability in models with a lower standard error of calibration but a higher standard error of cross-validation. Partial least squares regressions were found to be the most efficient method for calibration when processed with the "modified PLS" procedure of WinISI software (Win-ISI, Infrasoft International, Port Matilda, PA, USA). Reliability of prediction models was assessed by cross validation, with 6 subgroups representing the country of origin of the feed sample, resulting in the calculation of a standard error of cross-validation.

Results

Calibration equations (Eqa 1) derived from Set 1 (without the addition of individual AAs) are presented in Table 1. The concentration of methionine and cysteine are gathered into a unique variable (methionine + cysteine) since the nutritional significance comes from their sum as they are both sulphur containing amino acids which enter a similar metabolic pathway within the vertebrate body. The performance of Eqa 1, assessed by cross-validation for lysine and methionine + cysteine appears to be satisfactory when considering the repeatability of the reference AA analyses (Sr_{Lab}). However, there were always some outlier values (Figure 1a), where the value estimated by NIR spectroscopy was lower than the measured value. The measured values remained unchanged when the samples were re-analysed. The prediction of the ratio of lysine content divided by protein content (Figure 1b) was inaccurate. This means that the calibration of lysine content used generic protein information. When trying to predict independent samples after adding lysine (Set 2), the equations Eqa 1 failed completely (Table 2) with very high standard error of prediction values and all supplemented samples being prediction outliers. Also the ratio of lysine content divided by protein content could not be predicted as illustrated in Figure 2b.

Table 1. Calibration equation statistics on samples with no added amino acids (Set 1).

Constituent	n	Mean	SD	SEC	R^2	SECV	RPD	Sr_{Lab}
Crude protein (%)	127	17.3	2.83	0.46	0.97	0.64	4.42	0.35
Lys ($g.kg^{-1}$)	126	7.4	1.70	0.49	0.92	0.66	2.58	0.77
Met + Cys ($g.kg^{-1}$)	127	6.3	0.98	0.43	0.81	0.51	1.92	0.39

n: number of samples

SD: standard deviation of the population

SEC: standard error of calibration

R^2: coefficient of determination

SECV: standard error of cross-validation

RPD: ratio of performance to deviation ($SD.SECV^{-1}$)

Sr_{Lab}: repeatability of reference analysis

Table 2. Calibration equation statistics on samples with added amino acids (Set 1 and Set 2) and validation statistics on independent samples with or without AA added.

Constituent	Equation	n	SD	SEC	R^2	SECV	RPD	SEP on independent validation samples	
								Without AA added	With AA added
Lys	Eqa 1	126	1.70	0.49	0.92	0.66	2.58	0.67	2.70
	Eqa 2	228	3.37	0.75	0.95	0.91	3.70	0.69	0.79
Met+Cys	Eqa 1	127	0.98	0.43	0.81	0.51	1.92	0.66	2.43
	Eqa 2	227	2.98	0.65	0.95	0.86	3.47	0.78	0.94

n: number of samples

SD: standard deviation of the population

SEC: standard error of calibration

R^2: coefficient of determination

SECV: standard error of cross-validation

RPD: ratio of performance to deviation ($SD.SECV^{-1}$)

SEP: standard error of prediction

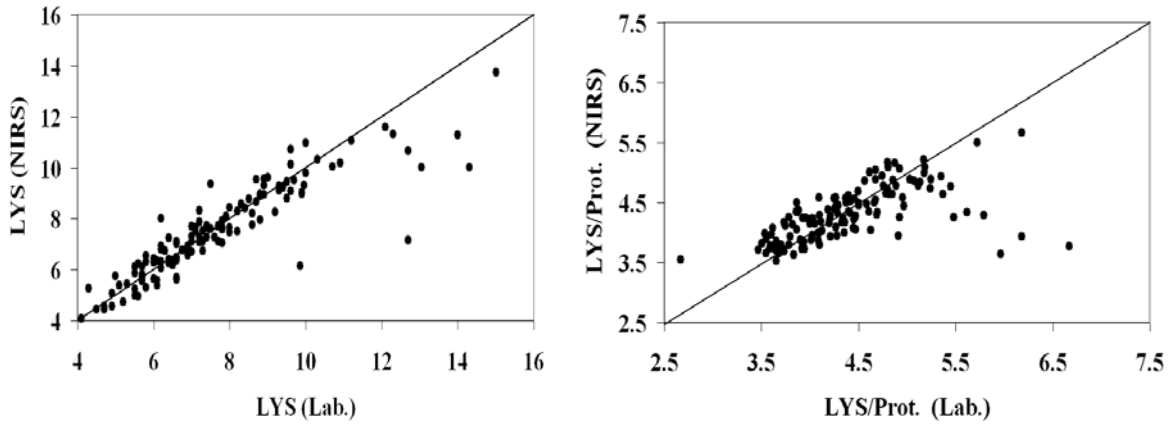

Figure 1. Plots of (a) lysine content and (b) lysine/protein content estimated by NIR spectroscopy against values measured by high performance liquid chromatography for samples with no added amino acids (Set 1, n = 130).

Equations Eqa 2, developed with Set 1 + Set 2, had higher standard errors of cross-validation than those of Eqa 1 (Table 2). This is partly due to the much higher variability of the database evident as indicated by the ratio of the standard deviation of the population divided by the standard error of cross-validation (RPD, ratio of performance to deviation). However, the SEP measured on independent samples without the AA addition of any individual AAs were comparable to those obtained with Eqa 1 (Table 2). When used to predict the AA content of independent samples to which AAs had been added, Eqa 2 equations performed better than Eqa 1 equations, with standard errors of prediction being very close to values for the standard error of cross-validation. The ratio of lysine content divided by protein content was also well predicted, as illustrated on Figure 2d.

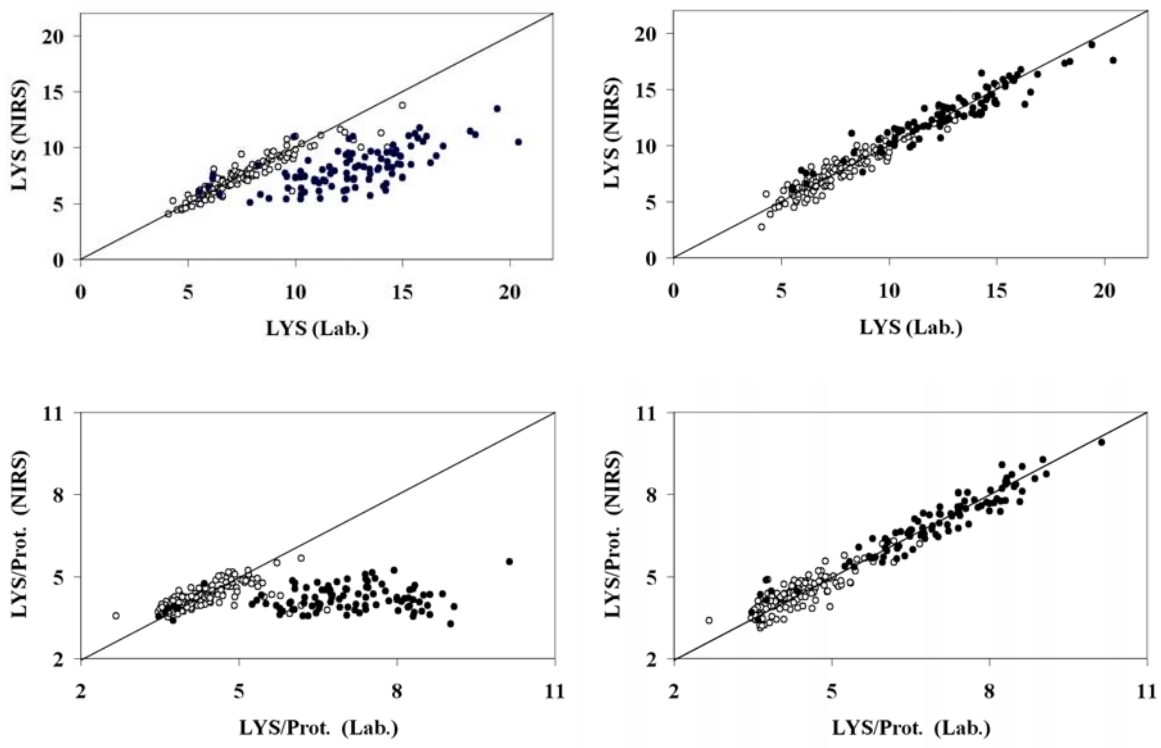

Figure 2. Plots of (a) lysine content and (b) lysine/protein content estimated by NIR spectroscopy using equations derived from calibration samples with no added amino acids (Equa 1) and (c) lysine content and (d) lysine/protein content estimated by NIR spectroscopy using equations derived from calibration samples with and without added amino acids (Equa 2) against values measured by high performance liquid chromatography, for the full set of samples (Set 1 + Set 2, n = 240).

Discussion

The method of "dosed additions" is well known in analytical chemistry in solutions but is seldom used in complex products [6]. In NIR spectroscopy it is used essentially in pharmaceutical industry [7]. It was used here because the purpose of the study was to develop models to predict the AA content of feeds with such additions, as are produced routinely in industry.

The use of databases containing samples with an AA added did not improve the prediction of samples containing no added AAs. Conversely, they allowed the prediction of samples containing a purified AA, which is impossible with Eqa 1 equations. Addition of pure AAs is common in practice in feed formulation, especially in contexts where protein-rich raw materials are expensive or imbalanced in AA content. Lysine, methionine and tryptophan are the AAs that are most widely used in this context. The prediction outliers observed in "natural feed" at the beginning of our study (Figure 1a) probably correspond to such feeds already supplemented with AAs by feed manufacturers. There are few such cases in the database because it concerns low quality feeds.

In fact, prediction equations built with databases without supplemented samples are based on generic protein wavelengths and to the correlation which exist between protein and individual AAs. This is why they are unable to predict AA/protein ratio which is independent from protein content with the relation between AA and protein often being linear. The AA/protein ratio is a relative proportion in that it is a characteristic of protein composition. The addition of a pure AA in the feed, with an appropriate experimental design breaks the AA-protein relationship, and the calibration equations developed in that way are based on information specific to a precise AA. In the present study, our attempts to interpret equation loadings to relate the wavelengths used to individual AA spectra were not successful. Also multiple linear regression models, which are not reported in this paper, performed badly compared to partial least squares regression techniques. This suggests that the pertinent information is located at several places in the spectrum.

Conclusion

It is concluded that NIR spectroscopy can be used for assessment of AA content in poultry feed with an accuracy that allows quality control. A very interesting point is that the strategy of including pure AAs leads to calibration models which are not based on AA-protein relationships and are therefore resistant to qualitative gaps between samples. The proportion of AA in proteins can also be predicted through use of AA/protein ratios.

Building this kind of AA calibration without adding any individual AAs requires a huge number of samples and would probably have failed in the context of our study on low quality African poultry feeds.

Nothing's magic: the precision will always be limited by the repeatability of the reference analysis, which is sometimes low for some AAs in some laboratories since accurate AA analysis is difficult to achieve under routine testing conditions.

Corresponding prediction equations are currently being transferred to local laboratories in East Africa [2].

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