# The use of near infrared spectroscopy to predict digestible amino acid contents of animal and vegetable protein sources for poultry nutrition

**J.C. Bodin and P.A. Geraert**
*Aventis Animal Nutrition, F-03600 Commentry, France.*

## Introduction

The animal feed industry bases feed formulation on supplying nutrient requirements at least cost (least cost formulation). Ingredient quality and prices vary greatly as a function of origin and market pressures and it is a recognised practice to formulate diets employing a safety margin in order to account for the variation in nutrient content of ingredients and to ensure that animal requirements are met. In the specific case of amino acids, when a safety margin is used for lysine, since all other amino acids tend to be formulated relative to lysine, all the others also increase. Thus, occasionally, safety margins may lower performance and exacerbate environmental problems, to say nothing of the additional cost. It has been demonstrated through formulation simulations that one of the keys to optimal feed formulation is accurate knowledge of nutrient composition and availability of the ingredients.[1] Classical methodologies to estimate amino acid contents and their digestibilities or availabilities are time-consuming and expensive as the experiments are *in vivo*.

In practical conditions in a feedmill, it is not technically possible to adjust ration formulations based on the nutritional profile of each batch of raw material received.

Currently, for raw material, the feed producer measures moisture, protein (N × 6.25), fat and ash contents of feedstuffs. However, these parameters are of limited value, since they only correlate to a limited extent with the true nutritional value of a feedstuff.

Concerning protein nutrition, the nutritional value of feedstuff depends on the quantity of essential amino acids available to the animal (bioavailability). The quality of a protein in feedstuff is determined by the net yield of amino acids from the feedstuff for metabolic processes and second, by the match of the net availability of amino acids with the requirements of the animal. Information on the digestibility of amino acids in feedstuffs has been provided to feed manufacturers in the form of tables which contain mean values for each feed raw material.

Although experience has shown that several different factors, such as heat and chemical treatment, anti-nutritional factors and environmental conditions, can influence the contents and digestibility of amino acids, these factors are ignored when a mean value is presented for each feedstuff. An example of feed raw material which exhibits high variability is animal meal. Coefficients of variation for nitrogen and the most important digestible amino acids are presented in Table 1.

The coefficients of variation are sufficiently high to limit the advantages of the mean values when attempting to evaluate accurately the nutritional value of feedstuffs. Depending on the origin of the feedstuff, the technical treatment used and the composition, it is difficult for the nutritionist to use an average value.

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**Table 1. Examples of coefficients of variation (%) for nitrogen, digestible lysine, methionine and threonine in meat and bone meals and fish meals.**

| Meat and bone meal | Fish meal |
|---|---|
| CV nitrogen = 10.1% | CV nitrogen = 8.7% |
| CV digestible lysine = 24.7% | CV digiestible lysine = 16.7% |
| CV digestible methionine | CV digestible threonine = 17.7% |
| CV digestible threonine = 19.5% | CV digestible methionine = 17.7% |

Near infrared (NIR) analysis applied to food products has developed since the end of the sixties.[2] Since then many instruments have been perfected and their main advantage is to allow rapid, direct and non-destructive measurements. Therefore, this method has many applications, particularly analysing raw materials.

From its first applications to determine moisture in soybeans, this method has become a valuable tool in the simultaneous measurement of protein, lipid, saccharose and fibre content.[3] The present study looks at the possibilities of determining total and digestible amino acids levels in animal and vegetable protein sources.

## Materials and methods

### *In vivo* digestibility tests

Aventis has been performing ileal digestibility tests since 1981 to estimate bioavailability of amino acids in feedstuffs using caecectomised cockerels. Digestion of proteins is complex, amino acids are absorbed in the small intestine. For poultry, caeca are equivalent to the large intestine and contain a significant microbiological activity which can alterate the measurement of the amino acids use. For this reason, caeca were removed by surgery as explained in Figure 1.

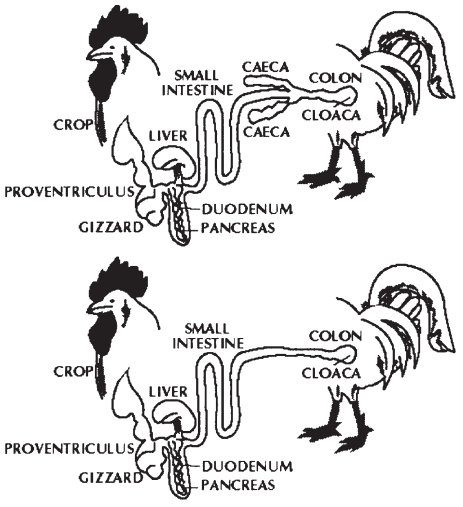


**Figure 1. Caecectomy principle.**[5]

The measurement of ileal digestibility of amino acids is recognised to be a valid tool for the estimation of amino acid availability. For the present experiment, animal meal and soyabean meal samples were tested using a procedure described in Reference 4. Birds were confined in individual wire-meshed cages, under which trays were fitted for excreta collection. During a test, birds were initially starved of solid food for 48 hours, during which time glucose was added to water bowls at a rate of 50 g every 24 hours for each bird. Each cockerel was removed from its cage and force fed 50 g of the experimental diet. Each experimental diet was a mix of wheat starch and feedstuff samples to be tested (animal meals or soybean meals) in different proportions in order to obtain an 18% fixed protein level always. 12 birds were used to evaluate each indi-

vidual feedstuff sample. Immediately after feeding, birds were replaced in their cages and all excretia voided during the following 48 hours were collected at eight-hour intervals. The amassed excretia were weighed and stored at 4°C. Excretia were pooled for four birds, freeze-dried and then analysed

## Chemical analyses and digestibility calculations

Raw material samples and excreta were analysed for nitrogen using the Kjeldahl method and for amino acids using a Beckman Multicrom amino acid analyser [High Pressure Liquid Chromatography (HPLC)] after hydrolysis for 24 hours with 6 N hydrochloric acid. For methionine and cystine analysis samples were subjected to performic acid oxidation before hydrolysis. In the specific case of excretia, uric acid was chemically separated from the excretia samples using the method described in Reference 6.

For each animal meal and soya bean meal samples, digestibility coefficients for nitrogen and amino acids were calculated and expressed as the ratio of the amino acids intake. Digestible amino acids contents were calculated using the digestibility coefficients multiplicated by the total amino acids levels determined by HPLC on each individual sample.

## NIR spectroscopy scanning and calibration procedures

Spectra from the animal meal and soya bean meal samples were obtained using an NIRSystem model 6500 (FOSS, Sweden). Before spectra recording, all the samples were ground through a 1 mm sieve using a Retsch ZM 1000 grinder. Spectral data were correlated with the total and poultry digestible essential amino acid data using NIR II version 3.00 (Infrasoft International, Port Mathilda, PA, USA). The partial least squares (PLS) regression technique was used. The spectral data were primarily subjected to a derivative math treatment 1,4,4,1 or a 2,5,5,1 (depending on the parameter studied) and a standard normal variates and detrending scatter corrections. These procedures allow us to obtain the optimal information coming from the spectrum and to reduce the particle size effect.

Two cycles of outlier eliminations were allowed, based on the spectral proximity or *H* value (*H* value larger than 4.00 = elimination) and the Student *T* value (*T* value larger than 2.50 = elimination). The spectral proximity is a statistical value which indicate if an unknown spectra is identical or close to a reference set of spectra. The student *T* value shows if a sample fits or not with a calibration model.

In a first step, performance of PLS calibrations were tested by cross-validation experiments. Cross-validation avoids the need to set aside samples for a validation set. In its original form, the idea was the following: one sample is dropped from the calibration test and an entire calibration is made

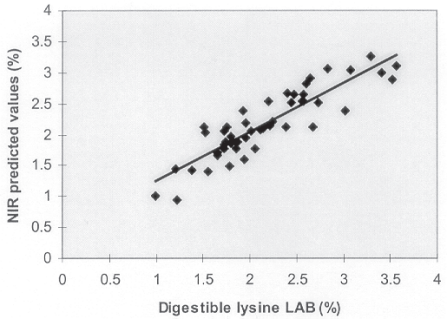


**Figure 2. Relationship between digestible lysine determined by the lab and by NIR predictions for meat and bone meals (g $100^{-1}$) as received.**

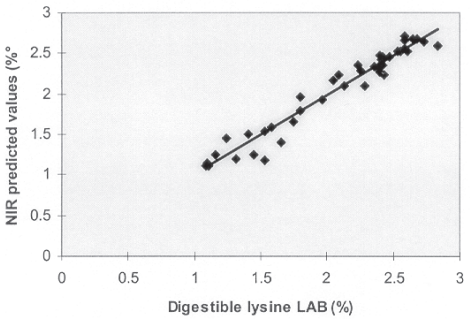


**Figure 3. Relationship between digestible lysine determined by the lab and by NIR predictions for soyabean meals (g $100^{-1}$) as received.**

**Table 2. Calibrations performance (g 100 $g^{-1}$ as received) for total and digestible, lysine—Lys and Lys(dig), methionine and threonine for animal meals, soybean meals and corn (*SECV* = Standard Error of cross-validation, $R^2$ explained variation, stdev = standard deviation of the reference population).**

| Meat & bone meals | Lys. | *Lys(Dig).* | Met. | *Met(Dig).* | Thr. | *Thr(Dig).* |
|---|---|---|---|---|---|---|
| mean | 2.67 | 2.16 | 0.81 | 0.69 | 1.76 | 1.39 |
| *stdev* | 0.65 | 0.60 | 0.21 | 0.20 | 0.38 | 0.34 |
| *SECV* | 0.23 | 0.27 | 0.09 | 0.10 | 0.13 | 0.16 |
| $R^2$ | 0.90 | 0.81 | 0.81 | 0.74 | 0.90 | 0.80 |
| *SECV*/mean | 0.09 | 0.12 | 0.11 | 0.14 | 0.07 | 0.11 |
| stdev/*SECV* | 2.83 | 2.22 | 2.33 | 2 | 2.92 | 2.12 |
| Fish meals | | | | | | |
| mean | 4.57 | 4.49 | 1.63 | 1.65 | 2.63 | 2.49 |
| stdev | 1.02 | 0.75 | 0.37 | 0.29 | 0.43 | 0.44 |
| *SECV* | 0.20 | 0.28 | 0.13 | 0.13 | 0.13 | 0.13 |
| $R^2$ | 0.96 | 0.94 | 0.88 | 0.81 | 0.90 | 0.89 |
| *SECV*/mean | 0.04 | 0.06 | 0.08 | 0.08 | 0.05 | 0.05 |
| stdev/*SECV* | 5.10 | 2.67 | 2.84 | 2.23 | 3.30 | 3.38 |
| Soya bean meals | | | | | | |
| mean | 2.36 | 1.93 | 0.61 | 0.54 | 1.61 | 1.31 |
| stdev | 0.63 | 0.68 | 0.08 | 0.08 | 0.26 | 0.27 |
| *SECV* | 0.15 | 0.15 | 0.04 | 0.04 | 0.08 | 0.09 |
| $R^2$ | 0.92 | 0.95 | 0.67 | 0.73 | 0.90 | 0.88 |
| *SECV*/mean | 0.06 | 0.07 | 0.06 | 0.07 | 0.05 | 0.07 |
| stdev/*SECV* | 4.20 | 4.53 | 2.00 | 2.00 | 3.25 | 3.00 |

with the remaining samples which is used to predict the sample left out. The standard error of cross-validation (*SECV)*/mean and stdev/*SECV* ratio were calculated to obtain an estimation of the predictive ability of the NIR calibrations. The difference between true and predicted value is used to calculate an average *SECV.* In a second step, two groups of samples coming from the field were used as validation sets to test the total amino acid calibrations and specially the total methionine prediction for soyabean meal and meat and bone meal. The difference between true and predicted values is used to calculate an average standard error of prediction (*SEP*).

## Results and discussion

The mean, standard deviation of the tested parameters, resulting from the the analysis of the samples and the statistical results of NIR spectroscopy calibrations for prediction of the most important total and digestible amino acids (lysine, methionine and threonine) are presented in Table 2.

Figures 2 and 3 graphically illustrate the relationship between laboratory determined and NIR spectroscopy predicted values of digestible lysine content in meat and bone meals, fish meals and soyabean meals.

Table 2 shows NIR calibrations explained 90% of the variation in total lysine, methionine and threonine for meat & bone meals and fish meals. For digestible lysine, methionine and threonine, mathematical models developed explained at least 85% of the variation. *SECV*/mean ratio obtained on total lysine, methionine and threonine varied from 0.04 to 0.11 against 0.05 to 0.14 for the same digestible levels. For soybean meals, NIR calibrations explained between 70 and 92% of the variation in total lysine, methionine and threonine and between 73 an 95% for the digestible contents. *SECV*/mean ratio for both total and digestible amino acids have an average value equal to 0.06. These ratios can be compared to HPLC average percent error which is close to 5%.

The above results show that NIR analysis is able to predict the total contents of the most essential amino acids in animal meal and soya bean meal. In both cases the lowest performances were obtained for total methionine. Two hypothesis can, therefore, be brought to the fore. First, methionine content in animal meals and soybean meals is approximately 50% less than lysine and threonine contents, thus the difference in accuracy can be linked to a sensitivity limit. Second, the reference range for total methionine level in the two feedstuff types is narrower than for lysine and threonine, which can explained the lowest performance of the developed model. The same remark can be applied to the digestible methionine calibrations which presented the same lack of precision in term of statistical results.

If we compare, on an overall point of view, the *SECV*/mean ratio for total and digestible amino acids, the performances obtain for total amino acids calibrations are always better than those obtained for digestible amino acids. It can be explained that the digestible amino acids reference values integrated the variability linked on one hand from the wet chemistry analysis (HPLC) and on the other hand from the *in vivo* test. However, if we take the ratio stdev/*SECV* as an indicator, several values presented in Table 2 are close, or equal, to the values which were considered adequate for screening (between 2.50 and 3.00) and for quality assurance (between 3.00 and 5.00) according to Reference 7. Thus, calibrations for total and digestible lysine, methionine and threonine may be used at least as a sucessful screening tool for the nutritional value of animal meal and soya bean meal.

This concept has been evaluated through the two validation groups (16 independent soyabean meals and eight independent meat and bone meal samples). More than a classical independent validation which commonly used 30 or more samples, these tests fit perfectly with practical conditions when a feed mill manager must deal with different suppliers and different quality for each type of raw mate-

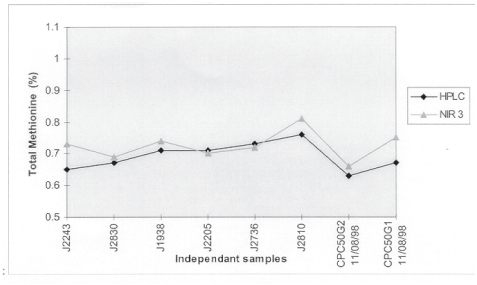


**Figure 4. Relationship between total methionine determined by HPLC and by NIR predictions for eight independent meat and bone meals from a feedmill (g 100$^{-1}$) as received.**

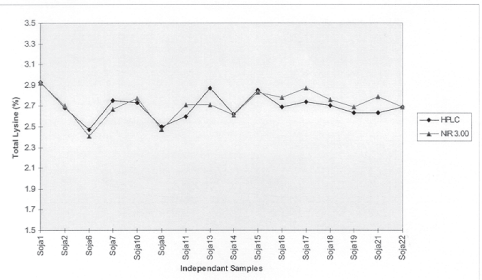


**Figure 5. Relationship between total lysine determined by HPLC and by NIR predictions for 16 independent soyabean meals from different customers (g 100$^{-1}$) as received.**

rial. Figures 4 and 5 represent the comparison between total methionine and total lysine NIR predictions and results obtained by HPLC analysis on eight meat and bone meal and 16 soyabean meal batches. The *SEP* obtained, respectively, 0.04 for total methionine on the eight meat and bone meals and 0.08 for total lysine 16 soyabean meal samples gives a ratio *SEP*/average lab value, respectively, equal to 8% for total methionine and 3% for total lysine. These results show that NIR estimations of total amino acids is as good as HPLC in estimating batch variation.

## Conclusions

It has been demonstrated that it is feasible to use NIR calibrations to predict total and digestible amino acid content in several important feedstuffs used in animal feed formulation . The $R^2$ and *SEP* for total and digestible amino acids were encouraging as an efficient screening tool to optimise feedmill management and feed formulation. These results were confirmed for total amino acids through the validations operated on two sets of samples coming directly from the field. This type of application illustrates the potentials of NIR as a rapid quality control tool well. Due to a continuously changing feedstuff market and because NIR is non-destructive and non time-consuming, it is completely adaptable to the time frame of feedmill management. NIR would thus allow feed producers to apply more relevant quality control which should allow the production of better quality diets.

With the appearance of new categories of raw materials (for example, high oil corn, soybean meals with high levels of lysine or methionine) it will be necessary to be able to measure the nutritional quality of crops rapidly on their arrival in the feedmill for storage and hence to re-adjust the feed-formulation.

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