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Reduction of nitrogen excretion in monogastrics. Improvement of precision in feed manufacturing

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SUMMARY - Animal feedstuffs are derived from multiple sources and exhibit a large variation in nutritional value. Techniques to quantify variation are not practical, being inaccurate and slow, and, hence, diets are more variable than desired. Reducing variation will improve the production efficiency at the mill and also limit nitrogen waste for pig and poultry units. The object of this paper is to compare the rapidity and the variability in the analysis of digestible amino acids in feedstuffs using existing methods, including regression analysis and *in vitro* digestibility measurements, with Near-Infrared Reflectance Spectroscopy (NIRS). An evaluation was undertaken using a theoretical feed containing 65% maize, 25% soyabean and 5% poultry by-products and the calculation of the coefficient of variation (CV) of nutrients, such as digestible amino acids, using the formula of Fawcett and Webster (1996). The CVs were 8.7 and 9.2% for digestible lysine and methionine respectively. Hence, assuming randomly-selected batches of feedstuffs, the feed was overformulated to a level of 107.5% for 80% of the finished feed to meet the animal's nutrient requirements. Existing techniques were shown to be unsatisfactory, failing to adequately predict the variation in digestible amino acids. However, the use of NIRS, a regression technique taking 5 minutes, is exhibiting potential. Calibrations with animal meals showed that 80 and 90% of the variability of the digestible methionine and lysine content respectively was explained, which was significantly better than other techniques. Overformulation was reduced to 103% in the example given above. Nitrogen waste, as calculated from the ratio of nitrogen excretion to accretion, was reduced by 13% as compared with randomly formulated feed. NIRS has been shown to be capable of being a practical accurate and rapid method of nutrient prediction which, potentially, could fulfil feed mill requirements. Work is continuing to expand the NIRS database.

Key words: Feedstuff, near-infrared reflectance spectroscopy, NIRS, digestible amino acids, pigs, poultry.

RESUME - *"Réduction de l'excrétion d'azote chez les monogastriques. Amélioration de la précision dans la formulation des aliments". Les matières premières utilisées dans la formulation des aliments pour monogastriques sont d'origines très diverses et présentent une importante variabilité en terme de valeur nutritive. Les techniques classiques permettant de mesurer ces variations s'avèrent peu pratiques, imprécises et lentes, ce qui, par conséquent entraîne une variabilité significative dans la composition des aliments complets. La réduction de cette variation permettrait d'accroître l'efficacité de production des usines d'aliments mais également de limiter les rejets azotés provenant des élevages porcins et avicoles. L'objectif de cette publication est de comparer la rapidité et la variabilité des méthodes d'analyse des acides aminés dans les matières premières (analyses de régressions, mesure de digestibilité* in vitro *vs NIRS, Near Infrared Reflectance Spectroscopy). Une évaluation a été menée en utilisant comme support un aliment théorique composé à 65% de maïs, 25% de tourteau de soja et 5% de sous-produit de volailles. Le calcul des coefficients de variation (CV) associés aux différents éléments nutritifs, tels que les niveaux d'acides aminés digestibles, a été réalisé à partir des formules de Fawcett et Webster (1996). Les CV étaient respectivement pour la lysine et la méthionine digestibles, de 8,7% et 9,2%. Dans ces conditions, les lots de matières premières étant prises au hasard, la formule a été surestimée à un niveau de 107,5% pour 80% du produit fini afin d'obtenir l'adéquation avec les besoins de l'animal. En ne permettant pas de prédire avec précision les variations des niveaux d'acides aminés digestibles, les techniques d'analyses classiques ont montré leurs limites. Au contraire, l'utilisation du NIRS, technique basée sur des régressions et ne prenant que 5 minutes par échantillon, a démontré son potentiel. En effet, grâce aux calibrations spécifiques des sous-produits d'origine animale, 80 à 90% de la variabilité des niveaux de méthionine et lysine digestibles ont pu être expliqués, ce qui est nettement supérieur aux autres méthodes. En comparaison avec la démarche de formulation précédente, la surestimation a été réduite à un niveau de 103%. Le niveau d'azote non utilisé, calculé à partir du ratio azote rejeté sur azote fixé, a été réduit de 13% en comparaison avec un aliment formulé de façon classique. La technique NIRS est apparue comme étant une méthode précise et rapide pour la prédiction de la valeur nutritionnelle. Des travaux sont en cours afin de développer la base de données NIRS.*

Mots-clés : *Matières premières, spectroscopie dans le proche infrarouge, NIRS, acides aminés digestibles, porcs, volailles.*

Introduction

Animal feeds are comprised of a range of ingredients, formulated at least cost, with the object of maximizing the performance of the animals to which they are given. On a world-wide basis many raw materials are used, of which large grain cereals such as maize, wheat and barley are major sources of energy. Primary sources of protein include soyabean meal, peas and fishmeal. However, cereals also provide important contribution of protein (amino acids). Feed formulation assures that these feedstuffs are mixed in specific ratios with the intent to create a final feed in which the optimum balance between the calculated amino acid and energy requirement of the animal is struck. In practice, however, diets are not always well-balanced due to variation in the nutritional quality of the feedstuffs. This then results in either energy being fed in relative excess to amino acids leading to undesirable accretion of fat, or amino acids being fed in relative excess to energy leading to an undesirable waste of costly amino acids. Feeding excess amino acids also augments the excretion of nitrogen, which is identified as one of the major pollutants derived from animal production. It is thus of prime importance for the feed industry to both understand the nutritional needs of the animals, as well as the nutritional value of the feedstuffs available to formulate feeds.

Feedstuffs are derived from multiple sources and exhibit a large variation in nutritional value. This variation poses a serious threat to efficiency given that techniques to quantify it are not practical, and are thus not used on a routine basis. Consequently, diets formulated using feedstuffs are inherently more variable than desired for optimal production conditions. To determine if this problem affects commercial feed mills, feed samples from two batches of the same feed from each of 12 different feed mills were analysed for their total amino acid content. The average difference in lysine content between the two batches was 7% for lysine, 14% for methionine, and 6% for threonine. Such differences are in strong contrast with the precision with which researchers attempt to define the nutritional requirement of animals, clearly indicating that techniques to quantify the nutritional value of feedstuffs on a routine basis are inadequate.

The result of the above problems with feedstuff evaluation is that feed companies frequently apply little quality control with respect to the nutritional assessment of the feedstuffs in the manufacturing of complete feeds. The overall objective of this paper is to review the limitations in the evaluation of the nutrient value of feedstuffs using existing methods, including regression analysis and *in vitro* digestibility measurements, and to compare the potential of Near-Infrared Reflectance Spectroscopy (NIRS) to meet these requirements. In particular, the consequence of variation in the digestible amino acid content between batches of feedstuffs on the quality of a final feed for pig and poultry units is addressed. The benefit that more accurate feedstuff evaluation may then offer in terms of reduced nitrogen excretion is discussed.

Evaluating the nutritional value of feedstuffs

The evaluation of the nutritional value of a feedstuff has been performed traditionally by analysing it for moisture, crude protein (N*6.25), fat, fibre, and possibly energy. These parameters are crude, particularly as the knowledge of the nutritional requirements of monogastrics has become relatively sophisticated. Certainly, evaluating the nitrogen content is inadequate (Wiseman and Cole, 1990). Today amino acid knowledge is vital for accurate formulation and the use of digestible amino acids levels is now accepted as a prerequisite in Europe for effective feed formulation. The measuring of amino acids is still a time-consuming and imperfect science. HPLC (high-pressure liquid chromatography), the reference method, is itself limited and involves corrections and an element of guesswork leading to inaccurate data (van Kempen, 1997). These problems are overcome in bio-availability assays, in which the feedstuff to be tested is a major source of the amino acid which is of interest. Lean tissue gain of the test animal is in such trials dependent on the quantity of the amino acid which is yielded (bio-available) from the feedstuff (Batterham, 1992). Although such tests provide the highest level of biological relevance, they are also the most complicated to perform and are not suitable for routine feedstuff testing.

A technique which is more practical is the ileal digestibility assay. Van Kempen (1997) pointed out that although it has suffered also from problems, this type of amino acid assay might solve the major discrepancy between amino acid availability and digestibility. The digestibility trials as currently performed with poultry yield data which in general predicts animal performance well (Han and

Parsons, 1990). In efficiently run laboratories, such a technique should allow for the evaluation of feedstuffs in approximately one week when working with poultry (requiring two amino acid assays, and approximately 1-2 man-days of labour). Such a turn-around time is suitable for occasional evaluation of the nutritional value of a feedstuffs, for example to update an ingredient matrix, but is not suitable for the routine evaluation of feedstuffs as needed in a commercial feed mill. This is unfortunate as it has already been shown that feedstuffs exhibit a large variation in nutrient supply between batches. Is it truly important?

Evaluating errors in formulation

The coefficient of variation (CV) of nutrients such as digestible amino acids in a complete feed can be calculated based on their variation of the individual feedstuff (Table 1) and the inclusion rate of these feedstuffs in the final feed, using the formula as published by Fawcett and Webster (1996). For a feed containing 65% maize, 25% soyabean meal, and 5% poultry by-products as the protein-containing feedstuffs, CVs for digestible lysine and methionine were calculated to be 8.7 and 9.2% respectively. A European-style diet of 13% wheat, 60% barley and 20% soyabean meal exhibited CVs for lysine and methionine of 7.8 and 6.4% respectively. The feed ingredients used influence the level of variability achieved but the principle holds for all complete feeds.

Table 1. Percentage content of digestible lysine and methionine and coefficient of variation between randomly selected batches of commonly used feedstuffs (data from the Rhodimet Nutrition Database)

	Dig. Lysine		Dig. Methionine	
	Content (%)	CV (%)	Content (%)	CV (%)
Wheat	0.25	9	0.16	15
Barley	0.32	13	0.15	8
Maize	0.19	10	0.15	18
Soyabean meal	2.44	10	0.55	11
Poultry by-products	2.22	34	0.77	27

Using these coefficients of variation, one can subsequently calculate to which level the feed should be overformulated such that, for example, 80% of the produced feeds meet or exceed the animal's nutrient requirement. Using the maize/soya example, assuming batches of feedstuffs were selected randomly, this value came to 107.4% for digestible lysine, while for methionine, it came to 107.8%. In other words, the feed comprising 65% maize, 25% soyabean meal, and 5% poultry by-products as the protein-containing feedstuffs, had on average an excess of 7.5% digestible lysine and methionine in order to meet the nutritional requirements of the animal for 80% of the feeds produced.

Various techniques have been developed with the objective to better quantify the digestible amino acid content of the batches of feedstuffs used to produce a final feed, and hence a reduction of the need to overformulate feeds. The effect that such techniques, provided that they are suitable for routine use, may have on the need to overformulate feeds is indicated in Fig. 1.

Do existing techniques allow for a fast and routine evaluation?

Regression equations

Attempts have been made to resolve the issue by performing simple analytical tests on the feedstuffs in order to yield values which can then be correlated with the digestible amino acid content of a feedstuff. Examples of such correlations (regression equations) can be found in many scientific papers, and often they are presented with regression statistics which make them appear very promising. However, too often the regression technique applied does not allow for the evaluation of the predictive ability of the regression equation, but only allows for the evaluation of the fit of the line through the data points available to generate the line in question. These two statistics are not the same (Esbensen *et al.*, 1996).

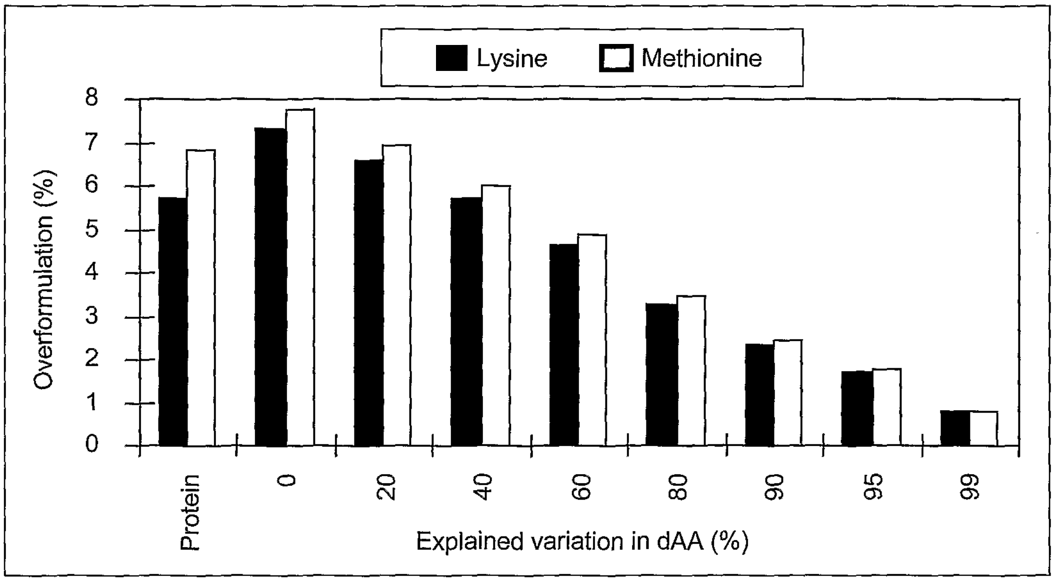

Fig. 1. Percentage overformulation required such that 80% of the feeds produced meet or exceed the calculated nutritional profile using batches of feedstuffs selected on their protein content. The calculations are based on a complete feed containing 65% maize, 25% soyabean meal, 5% poultry by-product, and 5% vitamin/mineral premix.

Van Kempen (1997) explained that regression equations using biological response parameters were often based on small databases, and the number of parameters used to explain the variability in the response variable was often larger than what is warranted by the size of the database. In such cases, data were likely to be overfitted. Variables might have explained variation within the database by chance, not because they described a causal relationship. Hence, they added no predictive value to the equation.

The problem may be circumvented by cross-validation. With cross-validation, a model is developed using all data points minus 1 (for small data sets; for large data sets they are split in, for example, 10 groups), and the developed model is used to predict the deleted data point. This procedure is repeated for all data points such that each data point has served as a control point. The difference between true and predicted values can then be used to calculate the prediction error of the modelling technique (the final model should be slightly better than the cross-validation models tested since the latter were based on less data points). Evaluation of regression equations should thus not be based on the r^2 of correlation, but on biological relevance (where applicable), and the r^2 of validation when comparing regression equations based on one database.

On this basis a more accurate evaluation may be made of the technique which has been used to predict the digestible amino acid content of a batch of a feedstuff: the determination of nitrogen. It has been assumed that the nitrogen content correlates with the digestible amino acid content. This hypothesis was tested using data from the Rhodimet Nutrition database (1993), for which nitrogen analyses were performed using the Kjehldahl method. True ileal digestible protein, lysine (dig. Lys) and methionine (dig. Met) were measured in poultry as per Green *et al.* (1987). Linear regression equations were determined using The Unscrambler Version 6.11 (a multivariate statistical analysis program from Camo AS, Trondheim, Norway) using full cross-validation in order to determine the explained variation (see Fig. 2 and Tables 2, 3, and 4).

It is evident that no statistically significant correlation was observed in maize between protein (nitrogen*6.25) and digestible lysine (Fig. 2). Summarizing similar information for soyabean meal and poultry by-products as well (Table 2) showed that protein did not correlate with the digestible lysine or methionine content for maize, while the correlations for poultry by-products were poor. For soyabean meal, the regressions were mediocre. Thus, knowing protein and using this information to predict the digestible amino acid content of a batch of a feedstuff would allow overformulation to be reduced to approximately 6.3%.

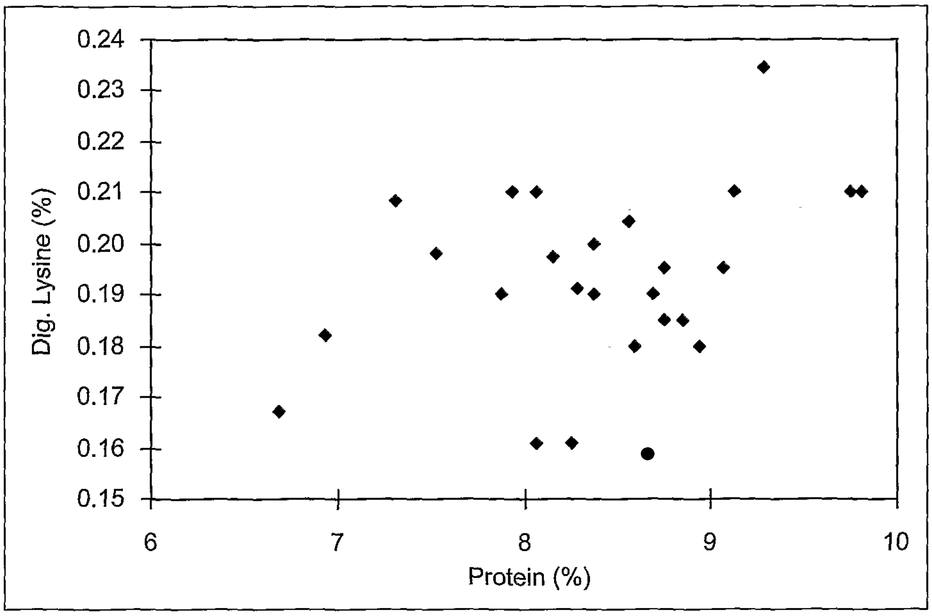

Fig. 2. Digestible lysine content (%) as a function of the protein (N*6.25) content (%) of randomly selected batches of maize.

Table 2. Variation (%) in true ileal digestible lysine and methionine explained by protein (N*6.25)

	Dig. Lys	Dig. Met
Maize	0	12
Soyabean meal	51	38
Poultry by-products	11	28

Including fibre data (NDF, ADF, and cellulose) to the regression equations improved the explained variation for plant products such that approximately 60% of the variation in digestible lysine and methionine could be explained (Table 3). Thus, knowing proximate analyses and using this information to predict the digestible amino acid content of a batch of a feedstuff would allow overformulation to be reduced to approximately 5.5% (for poultry by-product, the regression was still based on only protein). However, adding these parameters also increases the time required to complete the analyses needed for the regression. Fibre analyses performed using classical chemistry requires approximately two days, and are thus not suited for routine application.

In vitro digestibility measurements

An alternative method used to obtain information on the digestible amino acid content of a batch of a feedstuff is the *in vitro* digestibility assay. In such assays, either protein (nitrogen) digestibility or the digestible protein content is measured. The validity of the technique was tested using *in vivo* data rather than *in vitro* data (Table 4).

Table 3. Variation (%) in true ileal digestible lysine and methionine explained by protein (N*6.25) and fibre (ADF, NDF, cellulose)

	Dig. Lys	Dig. Met
Maize	59	56
Soyabean meal	56	55

The *in vivo* protein digestibility coefficients explained none of the variation in maize, while for soyabean meal and poultry by-products, in which heat treatment had an effect on the digestible amino acid content, a small portion of the variation was explained (Table 4). The regression lines obtained are, therefore, useless for practical application. The digestible protein content did not explain much variation in maize, and only a portion of the variation for both soyabean meal and poultry by-products.

Table 4. Variation (%) in true ileal digestible lysine and methionine explained by *in vivo* protein digestibility coefficients

	Dig. Lys	Dig. Met
Maize	0	0
Soyabean meal	35	27
Poultry by-products	33	2

A comparison of Table 5 with Table 2, in which crude protein was used to predict digestible amino acid contents, showed that the digestibility of protein added very little to the variation which was explainable for maize and soyabean meal, while a small improvement was noticeable for poultry by-products. The reason for this was that the *in vitro* digestibility assays ignored the fact that a large portion of the variation between batches of feedstuffs was linked to the amino acid content of a feedstuff, rather than to the digestibility of protein or protein content. To obtain useful information for routine feedstuff evaluation from *in vitro* digestibility assays it is thus necessary to determine both the amino acid content, and the amino acid digestibility. Consequently, this type of test is not suitable.

Table 5. Variation (%) in true ileal digestible lysine and methionine explained by *in vivo* digestible protein content

	Dig. Lys	Dig. Met
Maize	10	30
Soyabean meal	54	43
Poultry by-products	51	49

Current methods which yield nutritionally relevant data to evaluate feedstuffs are so cumbersome or costly that feed mills have not switched from the traditional measurements. As indicated above, though, apparently rapid indirect techniques are of limited value. It is clear that improvements in feed formulation can be made if rapid and reliable tools were available to assess the nutritional value of feedstuffs. One such technique which appears promising is the NIRS, or near-infrared reflectance spectroscopy. NIRS utilizes a principle which has been recognized for over 200 years: bonds between organic molecules absorb a specific wavelength range of light in the near-infrared region, and the near-infrared colour of a sample thus provides information about its composition. The functioning of NIRS and the underlying principles have previously been explained (van Kempen and Jackson, 1996).

NIRS is also a regression technique. Its predictions are based on correlations between spectral information (light absorption) and reference data (Shenk and Westerhaus, 1993) such as *in vivo* measured digestibility data. An advantage of such a system is that the ability to predict a response is not necessarily limited to our knowledge of the processes involved. This is important in, for example, heat treatment. Heat treatment will change the organic structure of materials, and thus the NIRS spectrum. Potentially, NIRS could use this information to correctly calculate the digestibility of amino acids of heat-treated materials (provided that such materials were included in the reference database). Such corrections are difficult to make when basing regression on proximate analyses results which do not necessarily change due to processing.

To test the possibility to obtain useful information about the nutritional quality of a feedstuff, Rhône-Poulenc Animal Nutrition has been working on developing NIRS calibrations for predicting the true ileal digestible amino acid content of a batch of a feedstuffs. For this purpose, feedstuff samples with known *in vivo* amino acid digestibility were scanned using a NIRSystems 6500 (monochromator instrument). Subsequently, spectral data from 1100 to 2500 nm were pre-treated with a standard normal variate and detrending scatter correction, and derivatised twice. Calibrations were then developed based on the converted spectral data using partial least squares regression. These calibrations were tested by running a cross-validation.

The first priority for the calibration development has been the prediction of animal meals since this is the category with the largest variation in digestible amino acid composition. The obtained prediction errors (RMSEP) and explained variation (r^2_{val}) for the three commonly used animal meals (meat and bone meal, poultry by-products, and fishmeal) for lysine and methionine are provided in Table 6.

Table 6. Calibration performance (g/100 g) of animal meal calibration 1.20 as applied to meat and bone meal, fish meal, and poultry by-products for poultry digestible lysine and methionine. (RMSEP=prediction error, r^2_{val}=explained variation as determined in the validation process)

	Mean	Stdev	RMSEP	r^2_{val}
	Meat and bone meal			
Lysine	2.29	0.64	0.22	0.88
Methionine	0.74	0.22	0.10	0.81
	Fishmeal			
Lysine	4.49	0.72	0.25	0.88
Methionine	1.65	0.28	0.11	0.86
	Poultry by-products			
Lysine	2.35	0.70	0.15	0.95
Methionine	0.80	0.18	0.08	0.80

It is possible to predict the digestible amino acid contents of animal meals since the NIRS predictions explained approximately 90 and 80% respectively of the variation in digestible lysine and methionine within the product categories (Table 6). Predictions for digestible lysine were more accurate than the predictions for methionine, which is probably related to the relatively lower levels of methionine in these products, and the higher assay variation for methionine. For the amino acids not shown, the calibration developed generally explained 70-90% of the variation within each of the animal meals presented.

Comparing the results in Table 6 with the results presented in Tables 2-4 shows that the NIRS was able to predict the digestible amino acid content of feedstuffs with a higher accuracy than current feedstuff evaluation techniques. Additionally, the NIRS results may be obtained in a fraction of the time it takes to determine proximate analyses or *in vitro* digestibility data (2-5 minutes with current equipment; in seconds with in-line equipment), and is thus ideally suited for routine tests. Assuming that NIRS calibrations can explain 80-85% of the variation in digestible amino acid content between batches of all important feedstuffs, overformulation of feeds may be reduced to 103% of the calculated requirements, from the original 107.5% (Fig. 3). It is obvious that such a reduction can result in savings on the side of feed preparation by reducing the inclusion of excess levels of nutrients.

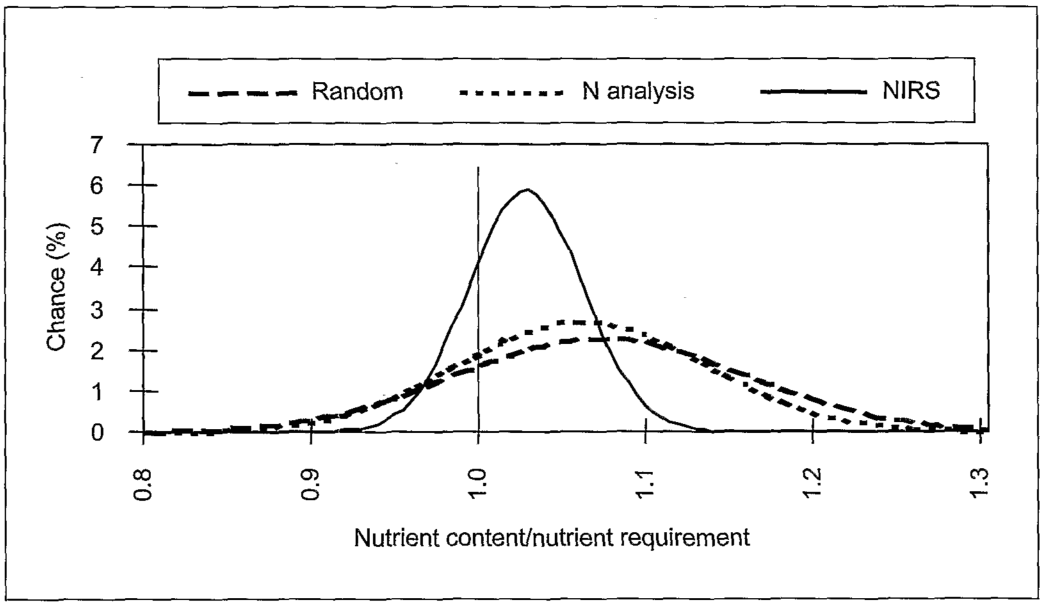

Fig. 3. Distribution of the nutrient (digestible amino acid) content relative to (calculated) requirement in feeds (based on 65% maize, 25% soyabean meals, 5% poultry by-products, and 5% vitamin/mineral premixes) formulated with: (i) randomly selected batches of feedstuffs; or (ii) with batches selected based on their protein (N) content; or (iii) with batches selected based on NIRS' predicted digestible amino acid content.

Small improvements in animal performance (lean tissue gain) should be obtained as well. The underformulated feeds (20% of the total) will still be closer to the intended feed if the feed is formulated with a higher accuracy (Fig. 3). For the feeds which were formulated either using randomly selected batches of each of the feedstuffs, or protein content, or NIRS-predicted digestible amino acid content, the average inadequate feed supplied 96, 96.9 and 98.5% respectively of the nutrient requirements.

Using the above information it is possible to calculate a theoretical effect of reducing overformulation on nitrogen wastage, and thus environmental pollution from broiler production. Additional assumptions for these calculations were that the feed conversion for the randomly formulated feed was 1.8. The feed itself contained 21% protein, while the birds gained 31.7 g N/kg feed consumed. The ratio of nitrogen excretion to nitrogen accretion then dropped from 0.91 for the randomly formulated feed, to 0.87 for the protein-formulated feed, and to 0.79 for the NIRS-formulated feed, a drop of 13% (reducing overformulation to zero would reduce nitrogen excretion to 0.66, a drop of 27%). Useful gains in production efficiency can thus be expected from formulating feeds with more accuracy.

The NIRS calibrations presented were a first effort based on a relatively small database, but clearly show that calibrations for animal meals are feasible. Preliminary work with soyabean meal and cereals has shown that it is possible to obtain calibrations for vegetable products as well. The limiting factor for vegetable products appears to be the ability to incorporate sufficient variability in the lysine content to provide enough of a range for the NIRS to recognize the regions of the spectra which correlate with lysine content. Targeted sample selection programs for *in vivo* tests for cereals, oilseeds, and animal meals are currently underway to expand the range, and thus the quality of the calibrations which may be derived from them.

The evaluation of the nutritional value of feedstuffs has long been based on nitrogen content, even though research has provided ample evidence that this parameter is not ideal. However, evaluation procedures which yield nutritionally relevant information have been too costly and/or time consuming for routine application, while the rapid techniques currently used are of limited usefulness. To resolve this problem, our research laboratory has tested if NIRS technology could provide a rapid technique to predict the digestible amino acid content of feedstuffs. These tests have shown that NIRS is capable of predicting these parameters more accurately than other rapid methods within a time-frame which fulfils the management requirements of a modern feed mill. The benefits gained from providing feed closely aligned to the animal's requirements will result in an increase in efficiency throughout the production system from the mill to the reduction of nitrogen waste off the farm.

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