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REVIEW ARTICLE

Use and Calibration of Near Infrared Reflectance Spectroscopy in Feed Analysis: A Mini Review

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ABSTRACT

The cost of feed in livestock and poultry farm operations is 65-70% which is major segment and has a direct influence on the profitability of farm. The feed formulation is a prime step which can result in low and high cost of feed. Use of low cost feed ingredients without compromising the quality of feed is the art of nutritionist at feed mills. Quality assurance holds the central role in formulating the good standard feeds for optimum production/yield. Quality analysis comprised of a series of tests which are based on wet chemistry analysis which are routinely performed in feed mills or at commercial livestock and poultry farms to check out the nutrient profile of feed and feed ingredients being used at the respective place to have an idea about the feed and its component's quality which have direct effect on the production of animals. Feed may contain inferior quality of nutrients or some anti-nutritional factors or toxins which can dramatically influence the health and production status of animals. So, the determination and quantification of these factors is very important on immediate basis to minimize the production losses. But the wet chemistry based tests are laborious, cost expensive and time consuming. The use of NIR Technology in feed laboratories seems best to overcome these challenges in the growing sector of livestock and poultry. The calibration of NIR is the major hindrance in its use. For this purpose, the following review has been written to take up the issue to develop an interest to explore the hidden characteristics of this technology in future and creating an innovation to use the NIR technology on commercial scale.

Contribution of Near Infrared Reflectance Spectroscopy in feed industry: an Introduction

Near infrared reflectance spectroscopy (NIRS) has shown to be a very fast, precise and non-destructive technique for routine analysis of various agricultural materials including forages (Norris et al., 1976; Sinnaeve et al., 1994). The use of Near Infrared (NIR) technology to determine simple components such as moisture, protein, fat, and fiber of major feed ingredients and finished feeds has been around for many years (Valdes and Lesson, 1994). However, the high costs involved in NIR equipment as well as the statistical expertise needed to calibrate, validate, and update equations in field conditions have been a major limiting factor in the wide use of this technology by industry. The feed industry requires rapid and accurate information concerning the nutritive value of feedstuffs. On one hand, such information is needed to negotiate the proper price for a feedstuff, and on the other hand, to correctly include this feedstuff in a complete diet such that the animal's nutrition requirements are met with the lowest feed cost. Preferably, such information is obtained rapidly so that a routine evaluation of a feedstuff is possible.

For adequate feeding of livestock, farmers need information about the nutritive value of available feedstuffs (Goedhart, 1990). Livestock selected for high production require an adequate supply of nutrients. This is essential not only for the health of the animals, but also from an economic viewpoint (Givens and Diaville, 1999; Givens et al., 1997). The wet chemical analyses of feed samples to determine their chemical composition are time consuming and expensive. Plant breeders, farmers and animal nutritionists require an accurate, precise, rapid and cost-effective method of assessing the nutritive value of pastures and feeds (Smith and Flinn, 1991). Near infrared spectroscopy (NIRS) provides an opportunity to determine the

chemical composition of feedstuffs. Apart from its rapidity, NIRS is a physical non-destructive method, requiring minimal sample preparation, with high accuracy. In contrast to traditional chemical analyses, NIRS requires no reagents, producing no waste. It is furthermore a multi-analytical technique as several determinations can be made simultaneously and once the NIRS is calibrated, it is simple to use and operate (Givens et al., 1997). For example, conventional chemical analysis of feeds will take two to three days, while a similar analysis can be completed in 2 - 3 minutes by NIRS (Corson et al., 1999). However, calibration sets with insufficient distribution of the samples could lead to inaccurate calibrations (Viljoen et al., 2005). Aufrere et al. (1996) stated that NIRS is not widely used for concentrates and compound feeds as a large number of samples are required for the calibration. Compound feeds are further spectrally complicated because of the wide choice of raw materials used in such feeds as an infinite number of combinations is possible (de Boever et al., 1995).

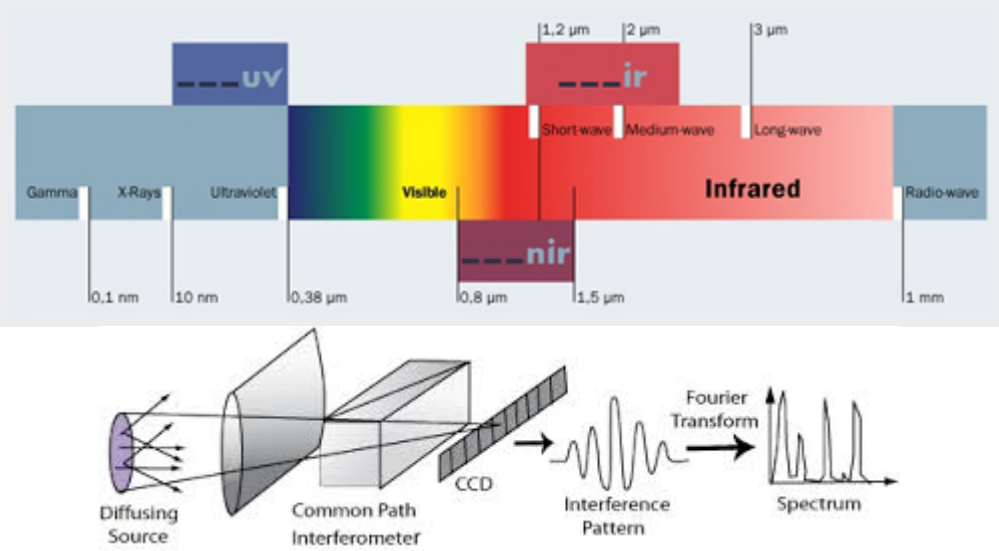
Currently, many feed mills use NIRS technology to predict the protein, moisture, fat, and ash contents of feedstuffs to obtain the needed information on a feedstuff. However, these parameters are of limited value since they only correlate to a limited extent with the true nutritional value of a feedstuff. Better parameters to evaluate are the amino acid availability, and the net energy of feedstuffs. Feedstuffs used in poultry nutrition are derived from multiple sources and exhibit a large variation in nutritional value. Most of the techniques used to quantify are not practical, being time consuming, slow and in some cases expensive, hence often final diets are more variable than desired. Reducing variation will improve the production efficiency in the mill and also the bird performances.

Overall, the animal feed industry is based on the search for the least cost formulation. Ingredient quality and prices may vary a lot as a function of their origin and the market. Several factors can affect the formulation but the 3 most important in balancing a diet are nutrient requirements of the animal, nutrient contents of the feedstuffs and nutrient availability. It is well known that there is a variation in nutrient content of ingredients and to be sure that the animal requirements are met, it is usual to provide a safety margin when formulating the diets. This is the case for vitamins but it is also used for protein. It has been demonstrated through different formula simulations that one of the principal ways to produce an optimal feed formulation is a good knowledge of the ingredients nutrient composition and availability (Bushman, 1998). On the world – wide basis and particularly in Europe, animal feeds are comprised of a more or less large range of ingredients. The different raw materials used are formulated at least costs with the goal to maximize the animal performances to which they are given. The objective of the feed formulation process is by the way to mix the different feedstuffs in order to obtain the optimum balance of nutrients to fit with the nitrogen and energy requirements. Deficiencies of the main nutrients like energy, proteins, amino acids, will get a direct impact on animal performances. On the other hand, relative excess of such nutrients will lead to possible accretion of fat and waste of amino acids which can increase nitrogen pollution impacts. Often, these nutritional deficiencies can cause measurable animal performances problems. For this reason, nutrients are often given a margin of safety for naturally occurring variance. But using a margin of safety does not reduce variability, which is the real problem (Van Barneveld, 2003).

The technique Near Infrared Reflectance Spectroscopy was first developed as a method of measuring water content in grains (Norris, 1964). This technique uses a principle which has been recognized for over 200 years. NIR is recognized by the Association of Official Analytical Chemists (AOAC) as an official method of analysis. Bonds between organic molecules absorb a specific wavelength range of light in the near infrared region, and the near – infrared color of the sample provides information about its composition. NIRS provides the ability to analyse feed ingredients at a cost much lower than and a speed much faster than conventional laboratory methods. NIR is calibration dependent. Traditionally, calibrations were developed for common feed types by analyzing several hundred to several thousand samples. The chemical information contained in these samples is correlated to spectral properties and a calibration is born. For example, a corn silage calibration is based on samples grown and harvested under varying climatic and geographic conditions. Calibrations built upon several years of information provide the power of being able to accurately analyze samples grown under a wide variety of conditions.

Comparison of wet chemistry analysis and NIRS

Wet chemistry analysis includes the traditional methods in which chemical are used and it takes long time. These include the Weende Analysis, Van Soest Partition of carbohydrates, Spectroscopy, Photometry and chromatography. No doubt these tests are reliable but are cost expensive and time consuming and can delay the inferential decision about the quality of feed and feed ingredients. While Near Infrared Reflectance Spectroscopy is a latest technology which is based on absorbance and reflectance of specific wave length. It can display results in less than 15 seconds when is operationally ready. In general for whole process of evaluation of feed sample including calibration with standard it may take upto 3 minutes. It is expensive but once purchased can work without running cost in an environment friendly way.



Graphical presentation of principle of NIR Spectroscopy (adopted from http://www.adphos.com/functionality_of_NIR_technology.html)

Calibration of NIR machine

The wet chemical analysis of feed samples is time consuming and expensive. Near infrared spectroscopy (NIRS) was developed as a rapid technique to predict the chemical composition of feeds. The prediction of accuracy of NIRS relies heavily on obtaining a calibration set which represents the variation in the main population, accurate laboratory analyses and the application of the best mathematical procedures. Swart et al. (2012) used NIRS to determine the chemical composition of total mixed rations (TMRs) used in ostrich diets. A sample population of 479 ostrich feed samples was used in the calibration and 94 samples were used in the independent validation of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), gross energy (GE), calcium (Ca) and phosphorus (P). Coefficient of determination in validation (*r*2v) and standard error of prediction (SEP) was satisfactory (*r*2v values higher than 0.80). Coefficient of determination and SEP values for CP, EE, CF, ADF, NDF and GE were 0.97% and 0.74%, 0.89% and 0.50%, 0.94% and 1.41%, 0.89% and 2.67%, 0.95% and 2.81% and 0.80% and 0.28 MJ/kg, respectively. Less accurate values (*r*2v below 0.80) were obtained for DM, ash, Ca and P being 0.57% and 0.28%, 0.67% and 1.29%, 0.43% and 0.59% and 0.49% and 0.11%, respectively. The study indicated that NIRS is a suitable tool for a rapid, non-destructive and reliable prediction of the chemical composition of ostrich TMRs.

Importance of NIRS use

While formulating ration along with other nutrients proteins especially amino acid content consideration is very important. Presence of balance and required amino acid in feed result in better performance. Total amino acid and digestible amino acids are determined by *in vivo* and *in vitro* techniques but these are time consuming and laborious. Generally most feed mills while formulating ration use some extra amount of digestible amino acid for the purpose of safety margin to avoid shortage of DAA in feed which may arise due to variation of DAA content in ingredients. But this over formulation results in increase feed cost and also increases N excretion which causes pollution. The calculation and prediction of the accurate amount of DAA amino acid content in ingredients can be done by NIR use if calibrated precisely with sufficient number of sample data (Jiang, 1999) and it will decrease the feed cost and balance amino acid profile. Ration formulation on the basis of digestible amino acid instead of total amino acid is more beneficial with reference to the performance of animals.

The near Infra Red Technology (NIR) has been used for proximate analysis in fee industry and its prediction are based on correlation (calibration curves) between light absorption in Infra red region and the chemical composition of test material. The development of precise calibration curve is the key to get accurate prediction. For calibration feed sample with predetermined values are scanned using monochromatic scanning NIR machine. Calibration curves were then developed based on pretreated spectral data from 1100 to 2500 nm using the partial least square regression (Van Kempen and Jackson, 1996) and then calibration curves were tested by running cross validation test and NIR was found able to explain 80-85% variation in dig methionine and lysine content in meat and bone meal.

Bodin and Aubert (2005) performed the calibration of NIRS and developed NIR calibrations to predict proximate analysis on a larger range of feedstuffs like pea, alfafa, rapeseed meals, sunflower meals, cereals bran. NIR calibrations explained between 80 and 98% of the variation for the overall proximate criteria, excepted for starch and fat in our corn data base.

Swart et al. (2012) used 479 TMR samples and analysed them with Weende Standard analysis and Van Soest and then compared and calibrated the values estimated by actual tests and NIR results. They stated that NIR is the best and quickest method if calibrated well with sufficient number of samples.

Feed quality is very important and has a critical role on performance. Quality includes nutritional contents and Hygiene parameters. For successful farming quality of feed with regard to nutritional value and hygiene should be focused. Nutritional value is maintained by analyzing ingredients with conventional instruments which may have some fluctuations and result in nutrients variation in feed and performance is compromised. To reduce the chances of fluctuation in nutrients contents in finished feed NIR is being used. For this purpose it is calibrated. Valdes et al. (1985) used Infra Alyzer 500 for the best set of wave length to calibrate CP, Ca and P in feed by using feed sample from 11 different laying hen operation units and accessed the accuracy of prediction of nutrients by standard error of estimate and by mean difference between chemical and NIR predicted values. NIR was found very reliable in predicting composition of nutrients after calibration. They declared that use of NIR result in analyzing feed ingredients more precisely and quickly to maintain quality standards and ultimately improved production performance.

Ramos and Madsen (2009) reported that two of the key methods available to understand the quality of the calibration have been shown to identify inaccurate NIRS calibrations that the ratio of standard error of cross validation to the standard deviation as well as the differences between the standard error of calibration (SEC), standard error of cross validation (SECV) and standard error of prediction (SEP) should be monitored in all NIRS calibrations. It is important that with careful attention to the process it is possible to produce reliable NIRS calibrations for the prediction of amino acid levels in feed raw materials

Zijlstra et al. (2013) developed NIR calibration of digestible energy for swine based on wet chemistry values of Regmi et al. (2009, 2008) and his calibration and validation statistics include the following accuracy for barley (SE of cross validation = 62.0 kcal/kg; R2 =0.88) and wheat (SE of cross validation = 72.1 kcal/kg; R2 =0.82).

The NIR was being used in Canada extensively but the issue with NIRS was producer confidence in the equations for our feed grains. Australian or European barley was viewed as different from western Canadian barley so there was a strong need to either modify existing or develop new calibrations to ensure accuracy and confidence. For this, Hand and Swift (2013) developed calibrations of 24 NIR machines which are being used in industry with confidence in results for measurement of moisture, protein, fiber (crude, acid, neutral detergent), ash, starch, lipid in barley, wheat, triticale, canola seed and meal, DDGS (wheat and corn), soybean meal, and DE (wheat and barley).

Forage carbohydrates account for 60-75% of total carbohydrates intake in dairy cattle ration. NDF and NFC suitable ratio in ration is very important to meet the energy requirement of animal and for normal rumen functioning. The estimation of these carbohydrates fractions for balance ration formulation is very tedious by conventional wet chemistry methods. Nie et al. (2009) used NIR for rapid prediction of carbohydrates fractions especially Neutral detergent Soluble carbohydrates (NDSC), scanning 1008 sample of Timothy and Alfalfa at 400-2500nm by NIR system 6500 monochromator and successfully predicted the concentrations of NDSC with coefficients of determination of prediction 0.93, and a ratio of prediction to deviation (RPD) of 3.6, respectively after calibrating it with results of wet chemistry analysis.

Feed intake of animal is very crucial to get required energy protein and other nutrients. Neutral Detergent Fiber (NDF) plays an important role in feed intake of animal. NDF is essential in maintaining the normal environment and functioning of rumen but if its concentration is high it will decrease the feed intake and affect the performance. For optimum production of animal, ration is formulated by calculating all the nutrients by chemical analysis and maintains them in a proper ratio including NDF and ADF but chemical analysis is time consuming and we can't perform tests on all the constituents ingredients as many ingredients are used in formulation having different composition which collectively contribute in overall composition of ration. Reeves (1994) made a regression equation for NIRS to check NDF and CP in treated forages and byproducts by using 325 representative samples for scanning and calibration of NIRS and get successful prediction of results with reliability on NIRS which is very quick and useful in ration formulation for the economic benefits of livestock.

Rao and Jones (2011) demonstrated the implications of NIR technology in feed industry for economic feed formulation to reduce cost based on balanced nutrients especially ME and DAA which are the real drivers of feed formulation. He pointed out that NIR technology is being used successfully in industry for quality control and quality assurance and it's giving reliable results with valid calibration which can also be cloned or transferred to other NIR Machines.

Wood and Badve (2012) compared the different techniques being used for feed analysis including wet chemistry and methods used to estimate anti-nutritional factors in feed. He stated that NIR technology is most reliable and quickest method for analysis and it is widely being used in UK but only limitation is the calibration and cost of instrument, once calibrated it is easy to use in predicting the composition of variety of ingredients including forages and concentrates feed for ruminant rations.

Use of NIRS in forestry

Gong and Zhang (2008) and Ren et al. (2008a,b) summarized the applications of NIRS to the field of forestry. The applications of NIRS in fruit quality, timber and seed quality analysis are more active in forestry due to its rapid, timely, less expensive, non-destructive, straightforward analytic characteristics. In the last two decades, non-destructive methods using near-infrared spectroscopy (NIRS) to evaluate parameters for estimating maturity were applied to different fruits species to check the ripening status of fruits on trees or to grade fruits in the packing house, to assess fruit quality, such as sugar and acid contents, soluble solids, firmness of fruit, offers great advantages to growers in deciding when to harvest. The near infrared spectrophotometry (NIRS) can also be used for the nondestructive quantitative assessment of the solid wood density, the moisture condition and the lignin content in bulky wood. The previous results indicated that the utility of NIRS was a selection tool in breeding programs, for example, three kids of persimmon fruits, astringent, non-astringent and half-astringent, were clearly classified by using Near-infrared (NIR) methods, and based on the combination of near infrared technology and multivariate analysis, the genetic, physiological and technical qualities of both temperate and tropical tree species on single seed basis can be characterized. It has already been shown that NIRS can predict the chemical composition of litters. NIRS is also capable of correlating the initial spectral characteristics of the litters with their short- and medium-term decomposability. The stage of decay of decomposing leaves can be predicted by using the near infrared reflectance spectroscopy.

Use in forage analysis

Forage was the material basis of animal husbandry production, and its quality is directly related to the quality of animal products. It was very important to control the forage quality and detect the composition of forage raw materials in forage production. Predication of forage quality was often completed by the traditional and classical methods in the past, which were complex, time consuming and expensive, and could not acquire the nutritional value of forage timely. Near infrared reflectance spectroscopy was a highly efficient and rapid modern analysis technique developed in 1970's. It comprehensively applied the latest research results of computer technique, spectroscopy and chemometrics, and has been widely used in various fields owing to its unique advantages such as being timely, less expensive, non-destructive, and so on. Near infrared reflectance spectroscopy has gained more and more importance though its application to forage analysis was very late. Presently, not only conventional composition (such as moisture, dry matter, crude protein, crude fiber, crude fat, crude ash neutral detergent fiber, acid detergent fiber, etc.), but also non-conventional composition (including minerals, trace elements, enzyme and anti-nutritional factors etc.) and anti-nutritional factors in forage were determined by means of near infrared reflectance spectroscopy. Testing and analyzing the conventional composition in forage was the traditional applied field of near infrared reflectance spectroscopy, a lot of studies of which were done and it has already been one of the standard methods of testing the conventional composition. Forage bioavaibility was also evaluated by near infrared reflectance spectroscopy, so as to assess the utilization rate and nutritional value of forage. Moreover, near infrared spectroscopy could be used successfully to predict the botanical composition in grassland and leaf/stem ratios.

Seed quality

Near infrared reflectance spectroscopy is a newly developed method capable of analyzing the content of specific compound in the detected sample quickly and efficiently. Near infrared reflectance spectroscopy has been wildly used in many fields such as agriculture science, food industry, medical industry, chemical engineering and so on. Based on this introduction, the potential value of the application of near infrared reflectance spectroscopy to the grass seeds quality certification was discussed in the four areas, i.e. the grass seed variety discrimination, the standard percentage rate of grass seed germination testing, the scale of the content of grass seed moisture, and the evaluation of the grass seed vigor and the purity of grass seed. Finally, it might be concluded that the application of near infrared reflectance spectroscopy to the grass seed quality certification is significant both in the academic and the technical areas because near infrared reflectance spectroscopy will not only improve the efficiency of grass seed certification, saving manual work and testing time, but also help expand the extent and application of routine quality certification of grass seeds.

New areas for NIRS use

Toxins and anti-nutritional factors are the major constraints which are causing major havoc in decreasing the production of livestock and poultry especially in dairy cattle and broiler production industry. These toxins affect the animal in chronic way and impose non compensatable losses as the detection

of these factors takes a long time by traditional methods. If NIR is used in detection of these toxins it can solve the issue in very less time and can result in better production.

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