NITROGEN CORRECTION OF TRUE METABOLIZABLE ENERGY VALUES FOR POULTRY FEED INGREDIENTS AND THEIR PREDICTION BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

by

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(Under the Direction of Adam Davis)

ABSTRACT

Feed accounts for around 70% of poultry live production costs, therefore, knowing nutrient availability in poultry feed ingredients is critical to maximize feed efficiency in poultry. One of the most important nutrient specifications to have for poultry feed ingredients is the nitrogen corrected true metabolizable energy (TME_N). However, the animal bioassay to determine TME_N is expensive, and results are not available for at least two weeks. Near Infrared Reflectance Spectroscopy (NIRS) is a rapid analysis method that has been utilized successfully to predict nutrient concentrations in feed ingredients but using this method to predict digestible nutrient values such as TME_N has not been validated. The accuracy of a constructed NIRS calibration curve to predict TME_N in feed ingredients would be heavily dependent on the accuracy of the rooster TME_N bioassay values from which it was built. The TME_N bioassay and associated calculations were developed over 40 years ago, and the assay was not validated for many currently utilized poultry feed ingredients. In the current research, TME_N values were determined using the rooster bioassay on over 700 ingredient samples across more than 50 different ingredient types to determine if the original bioassay procedures and calculations

developed with 13 feed ingredients and 24 samples were appropriate for a diverse array of poultry feed ingredients. While the bioassay procedure was proven appropriate for all samples tested, the original nitrogen correction calculation associated with determining the bioassay TME_N value was proven to be inappropriate for about 10% of the samples tested. After adjusting the calculation for these samples, an NIRS calibration curve to predict TME_N was constructed for all feed ingredients, and then validated with 551 different ingredient samples. The NIRS predicted TME_N values deviated 10% or less from the corresponding bioassay determined values for 97% of the validation samples. However, when ingredient specific NIRS calibrations were made to determine TME_N in bakery meal, soybean meal or corn the NIRS predicted values all deviated less than 2.5% from their bioassay determined value. The results indicate that NIRS can predict the TME_N of poultry feed ingredients accurately.

INDEX WORDS: Feedstuff evaluation, Digestibility, Alternative ingredients, Feed formulation

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CHAPTER 1

INTRODUCTION

Poultry diet ingredients

Global food demand has been predicted to double in the next 50 years (Falcon et al., 2022; FAO, 2022). This projected growth is greater than the forecasted increase in global food production (Tilman et al., 2011; Falcon et al., 2022; FAO, 2022). Poultry meat and egg production have had the highest growth rate during the last 50 years of any animal protein consumed by humans (Mottet & Tempio, 2019). This growth is mainly due to its affordability when compared to other meat derived proteins (Baldi et al., 2021). Because modern poultry production has become more efficient when compared to other livestock such as pigs and cattle (Leinonen & Kyriazakis, 2016), to meet the growing food production demand, the poultry industry will likely play a substantial role in ensuring global food security.

Maintaining production efficiency in poultry is heavily dependent on providing cost effective diets, which match nutrient requirements for growth and egg production while limiting nutrient excesses beyond what is required. Feed accounts for around 70% of live production costs, with feed ingredients providing energy and protein being the majority of those costs (Olukosi et al., 2017; Zampiga et al., 2021). In general, poultry diets are composed of a cereal grain such as corn or wheat to provide energy, a concentrated source of protein such as soybean meal and/or animal by-product meal, and a fat/oil to provide additional energy, increase palatability and reduce dustiness of the feed. These main ingredients are supplemented with synthetic amino acids, vitamins, and minerals to make a diet that meets the bird's nutrient

requirements. Because the cereal grains and soybean meal used in poultry diets are also used to feed the growing human population, poultry feed costs will continue to increase. To combat this competition for feed ingredients, alternative feed ingredients for poultry production are often utilized.

Alternative ingredients

Utilizing regionally available alternative feed ingredients can often lower feed costs. Alternative ingredients are typically ingredients that have not been previously used on a regular basis. For example, dried distiller's grains with solubles (DDGS), a by-product from ethanol fuel production and alcoholic beverage production has a high protein and energy content that is between cereal grains and soybean meal, and thus can be added to poultry diets at 10% or less without affecting performance as reviewed by Lumpkins et al. (2004; 2005a; 2005b) while decreasing the corn and soybean content of the diet. Peanut meal is another high protein alternative ingredient that is commonly used. Peanut meal has around the same protein content as soybean meal after oil extraction and can be incorporated into poultry diets (Dale, 2004). Sunflower meal is also gaining popularity for its use in poultry diets as a protein source. It contains about 25-30% crude protein and is free of anti-nutritional factors that are generally found in soybean meal (Senkoylu & Dale, 1999; Alagawany et al., 2015). A popular alternative energy source is bakery meal. This meal is a mixture of unsalable waste products from the baking industry. It is high in energy, easily palatable, and can be incorporated up to 10% in poultry diets (Damron et al., 1965; Saleh et al., 1996). However, when utilizing these alternative ingredients, it is imperative to have determined their nutrient specifications, as differences in the processing of DDGS, sunflower meal, and peanut meal and the composition of bakery products can significantly alter their nutrient content (Waldroup et al., 1982; Dale et al., 1990; Dale, 1992;

Senkoylu & Dale, 1999; Lumpkins et al., 2004; Batal et al., 2005; Batal & Dale, 2006; Senkoylu & Dale, 2006).

Consumer driven preferences in poultry production are also impacting poultry diet formulation and increasing the demand for determining ingredient nutrient content. For instance, organic poultry products require the use of organically grown feed ingredients that must be free from insecticides, synthetic fertilizers, and cannot be processed with certain chemicals (USDA National Organic Program, 2008). This means that poultry nutritionists cannot use solvent extracted soybean meal or any feedstuffs processed or extracted by chemical means. Oilseed meals produced by mechanical extraction methods have a different nutrient composition and nutrient digestibility coefficients than solvent-extracted meals (San Juan & Villamide, 2001; Iji et al., 2017). In addition, the organic specifications limit the use of chemically produced synthetic amino acids. For example, the organic specifications limit the use of commercially available synthetic methionine to 2 pounds per ton for layers and 2.5 pounds per ton for broilers (USDA National Organic Program, 2008). However, cereal grain and soybean meal based diets are deficient in methionine relative to the requirement of this essential amino acid in poultry (Jacob, 2013). Thus, nutritionists must find organic ingredients that are high in methionine such as fish meals and sunflower meals to formulate diets that meet the requirements of the birds they are feeding. Also, since pesticides or herbicides cannot be used, organically grown plants have developed mechanisms to protect themselves from pests, which in turn, reduces their nutrient digestibility by increasing the levels of antinutritional factors such as non-starch polysaccharides (Moritz et al., 2005; Buchanan et al., 2007).

Poultry products produced using all vegetable diets have also gained popularity with the consumer. This type of diet is composed of only cereal grains and vegetable protein meals and

has no animal residues included (Vieira & Lima, 2005). Animal by-products have a very high protein content, are often a source of highly digestible minerals such as phosphorus and calcium and lack indigestible complex carbohydrates. Thus, nutritionists formulating exclusively plant based poultry diets have to combat with the amount of indigestible carbohydrates and antinutritive compounds found in such diets (Kocher et al., 2003). This has led to an increase in the use of exogenous dietary enzymes such as phytase and carbohydrases, in all vegetable diets to liberate minerals complexed to phytic acid and to increase carbohydrate digestibility (Erdaw et al., 2016; Aftab & Bedford, 2018). Typically, the exclusion of the protein content found in animal by-products is alleviated by increasing the dietary level of soybean meal, but as mentioned previously, because soybean meal is also a food source for humans, increasing its demand and price there is pressure to find soybean meal alternatives such as peanut meal and sunflower meal.

Antibiotic free refers to the production of poultry in which there is no use of antibiotics for growth promotion, feed efficiency, or disease prevention (Cervantes, 2015). Due to concerns about antibiotic resistance, limitations regulating the use of antibiotics in poultry production have been increased (United Sates Food & Drug Administration, 2015). Additionally, in order to meet customer demands, poultry integrators have reduced the use of antibiotics in their production systems. However, it has been reported that the use of antibiotic free diets diminishes broiler feed efficiency and can increase disease incidence and mortality (Emborg et al., 2001; Smith, 2011). Therefore, a growing number of feed ingredients with nutraceutical properties deemed as antibiotic alternatives has emerged. These feed additives include probiotics, prebiotics, yeast components, organic acids, and plant extracts to name a few (Applegate et al., 2010). The

nutrient composition and availability of these nutraceutical ingredients is needed for accurate diet formulation.

Dietary composition of standard ingredients

The nutrient composition of cereal grains and soybean meal also need to be constantly assessed. The chemical composition and nutrient availability of soybeans and cereal grains vary with agronomic and processing conditions (Waldroup et al., 1985; Parsons et al., 1992; Choct & Hughes, 1997; Rowe et al., 1999; Palacios et al., 2004; Goldflus et al., 2006). In addition, seed companies continually use genetic selection to develop and introduce new varieties of cereal grains and soybeans with favorable characteristics. For example, due to the rise in biodiesel production, high oil soybeans are being grown over other types (Clemente & Cahoon, 2009). High oil grain varieties for corn and sunflower meal are also being produced (Saleh et al., 1997; Senkoylu & Dale, 1999). Finally, genetically modified varieties of cereal grains and soybeans are being developed and introduced to improve such attributes as insect resistance and methionine content (Chesson & Flachowsky, 2003; Swiatkiewicz & Arczewska-Włosek, 2011) and the nutrient composition and availability of these varieties must be assessed prior to poultry diet formulation.

Storage conditions also play a role in the nutrient composition of cereal grains and soybean meal. For example, mycotoxin levels increase in corn if they are stored in suboptimal conditions (Ogbuewu, 2011), with temperature and humidity during storage playing a critical role. Excesses of either can contribute to mold growth and the production of mycotoxins, which are harmful when ingested and decrease the quality and digestibility of the feedstuff (Ogbuewu, 2011). Time in storage can also impact the nutrient composition, specifically the metabolizable

energy of grains. One study found that after 3-4 months proper storage, the metabolizable energy of new season grains actually improved (Choct & Hughes, 1997).

Energy determinations

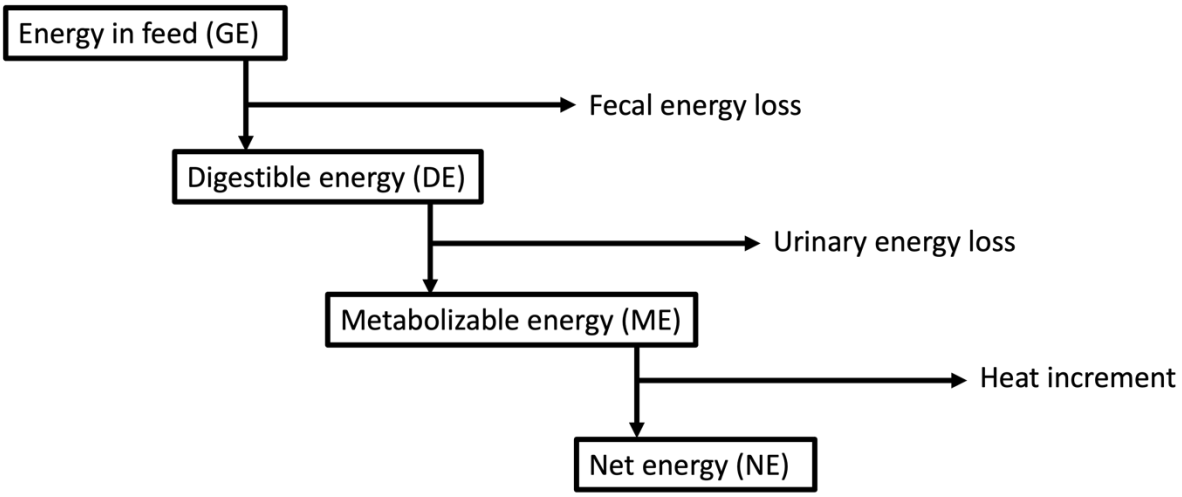
For poultry nutritionists, a measure of the metabolizable energy and digestible amino acids are among the most valuable nutrient specifications to have for the primary feed ingredients, as it is the available energy and digestible amino acid content that will fuel and sustain the rapid growth of broilers and the prolific egg production of hens for the table egg market and for breeding purposes. This review will focus on energy determination of feed ingredients, as the importance of digestible amino acids in diet formulation has been recently reviewed by others (Bailey, 2020; Parsons, 2020; Kidd et al., 2021).

Energy is not a nutrient, but rather a measure of the energy yielding nutrients of a feed ingredient when these nutrients are metabolically oxidized by an animal. The energy derived by the bird can then be utilized for maintenance and growth and/or production. Multiple energy measurements have been defined for feed ingredients.

Gross energy (GE) is defined as the total chemical energy released during the complete oxidation of a feed ingredient into carbon dioxide and water (Leeson & Summers, 2001). Gross energy is determined using a bomb calorimeter which measures the heat produced during the combustion of the feed ingredient. However, GE values for feed ingredients are not that useful, as the amount of the gross energy available to the bird is dependent on how much of the GE can be digested (Figure 1.1).

Apparent digestible energy (DE) is calculated by subtracting the fecal energy excreted from the gross energy of the feed ingredient consumed (NRC, 1994). However, this value is typically not used when formulating poultry diets because the feces and urine are mixed in the

Figure 1.1. Energy partitioning diagram



cloaca and excreted together in the bird. Therefore, apparent DE cannot be determined without surgical modification (Sibbald et al., 1962) or by the collecting of ileal digesta. Using the latter approach, apparent ileal digestible energy is determined by collecting ileal contents from the distal two-thirds of the ileum immediately after euthanasia (Sibbald et al., 1960; Bolarinwa & Adeola, 2012; Cao & Adeola, 2016). However, this value does not account for the microbial digestion occurring in the ceca that contributes energy utilized by the bird. Thus, the energy that is available to birds when formulating diets is better described by using metabolizable energy values (Hill & Anderson, 1958; Potter & Matterson, 1960; Potter et al., 1960; Lopez & Leeson, 2007, 2008a).

Since its acceptance, the assessment of metabolizable energy (ME) has become the first step in feed ingredient evaluation for poultry nutritionists (Hill & Anderson, 1958; Abdollahi et al., 2021). There are two commonly used measurements of ME, and they are apparent metabolizable energy (AME) and true metabolizable energy (TME). The gross energy of the feed ingredient consumed minus the gross energy contained in the feces, urine, and gaseous products of digestion equals the AME (Hill & Anderson, 1958). In poultry species, with negligible gaseous products from digestion, AME is a more logical measure than apparent DE because fecal and urine wastes are combined in poultry excreta.

A TME value of an ingredient is calculated by subtracting the gross energy of the excreta of feed origin from the gross energy of the feed ingredient and is a further refinement of the AME procedure. The TME procedure differs from the AME procedure, as it was developed to account for the endogenous energy loss in the excreta (Sibbald, 1975, 1976; Dale & Fuller, 1984). Not only does the excreta contain the nondigestible components of the feed/ingredient, but it also contains sloughed intestinal cells and gastrointestinal secretions (endogenous

component) produced by the bird. By correcting for the endogenous component of the feces, the digestible component of the ingredient is not penalized for the endogenous component of the feces, which ensures a more accurate energetic value is obtained (Harris, 1966; Sibbald, 1976). Typically, a nitrogen (N) correction factor is applied, as will be further discussed, to yield AME_N and TME_N values and in poultry, and TME_N and AME_N values are typically used for feed formulation (Wu et al., 2020).

Although ME is the standard measurement used to reflect the energy available to the bird, it is not fully reflective of the energy available for maintenance, growth, and/or egg production (Sibbald, 1982; Noblet et al., 1994; Pirgozliev & Rose, 1999; van der Klis & Jansman, 2019; Wu et al., 2019). Net energy (NE) is defined as the metabolizable energy content minus the heat increment. The heat increment represents heat loss (energy) due to nutrient digestion and assimilation. The determination of NE involves placing the animal in a metabolic respiration chamber to allow the measurement of the energy losses in the form of heat and gas. This method is costly and labor intensive and a limited number of animals can be used for the study (Kong & Adeola, 2014). Also, the determined value varies with the stage of growth of the animal (Noblet, 2015). Very few feed ingredients have calculated net energy values due to the cost and variance. Thus, ME values rather than NE values are most commonly used for poultry diet formulations (Hill & Anderson, 1958; Pirgozliev & Rose, 1999).

AME_N determination

The methods used for AME determinations of feed ingredients are the direct method, the substitution method, and the regression method (Adeola, 2000; Abdollahi et al., 2021). In all three methods for AME determination, four to six chicks are typically used per replication, with three to six replications typically used per experimental diet (Hill & Anderson, 1958; Olukosi et

al., 2017; Abdollahi et al., 2021). The birds utilized in each method can be age and type specific making determination very relevant to formulating specific diets. However, most of the research uses broilers between 7 and 49 days of age, as broiler production is the largest segment of the poultry industry.

In each AME determination method, excreta collection can be performed two ways, either by total collection or partial collection (marker method). In total collection, all excreta produced by the test birds is collected over the test period (Dourado et al., 2010). In partial collection, a known amount of an indigestible marker such as chromium oxide is added to the assay diet (Cortés et al., 2009). Excreta is collected, and the AME is calculated using the laboratory determined concentration of the marker in the feces and in the diet as part of the calculation (Sales & Janssens, 2003; Dourado et al., 2010). Also in each of the AME determination methods, birds are usually fed a standard commercial diet for the first 14 days after hatching before being administered the experimental diets (Olukosi et al., 2017; Olukosi, 2021). The experimental diets are typically fed for 2-14 days, with 4-7 days being the most common practice, before excreta collection begins, in order to adequately acclimate the test animal to the diets (Hill & Anderson, 1958; Potter et al., 1960; Lockhart et al., 1967; Meloche et al., 2014; Ullah et al., 2016; Latifi et al., 2023). This aids in minimizing any properties of the test diet that may affect the rate at which nutrients pass through the gastrointestinal tract. One study found that there was no significant effect between a 4, 7, or 10 day adaptation length for broilers (Olukosi et al., 2017). After the adaptation period concludes, typically, between a 48-to-96-hour experimental period commences when feed intake is monitored, and feces is collected.

The direct method is commonly used when determining AME values for cereal grains. In this method the test diet incudes only the ingredient being tested along with the macromineral

sources and the vitamin and trace mineral premixes. Some limitations for this method are that it cannot be used for poorly palatable ingredients, as intake of the diet is free-choice, and because the diet does not meet all nutrient requirements, adverse effects on body functions may be seen when fed for extended periods (Wu et al., 2020; Khalil et al., 2022).

The substitution method is an alteration of the direct method of AME determination, and it is typically used for ingredients with high protein content and/or poor palatability. A reference diet and an assay diet must be formulated as part of the substitution method. The reference diet is typically a standard corn-soybean meal diet whereas the assay diet is formulated by replacing a percentage of the reference diet with the test ingredient. There are two approaches used for the substitution method. In the single ingredient replacement approach the inclusion of a well characterized ingredient such as glucose is replaced in the reference diet by the test ingredient in the assay diet. In the complete basal replacement approach the test ingredient in the assay diet is added at the expense of a portion of all the major energy contributing ingredients of the reference diet. Both approaches minimize the potential for nutrient deficiencies and diet refusal seen with the direct method (Kong & Adeola, 2014). One disadvantage of this method is that the AME of the test ingredient can be influenced by the composition of the basal diet (Sibbald et al., 1960). Another is that the inclusion level of the test ingredient can significantly affect the AME value (Olukosi, 2021). For example, one study concluded that the AME determination became sufficiently accurate at inclusion levels of 300g/kg or more for the feedstuffs tested (Olukosi, 2021).

The regression method was first introduced by Potter et al. (1960) and utilizes the same principles as the substitution method; however, there is a minimum of two assay diets, each containing a different level of the test ingredient. The energy value is then calculated for the

individual assay diets and then compared to their corresponding inclusion levels and energy contributions of the test ingredient. The ME intake (kcals) from the test feed ingredient is regressed against its feed intake (Duodu et al., 2003) with its slope equal to the ME (kcal/Kg) of the test feed ingredient (Kong & Adeola, 2016). The regression method, based on the data from multiple inclusion levels of the test ingredient, reduces the influence of test ingredient inclusion level that is seen with the substitution method (Kong & Adeola, 2016; Abdollahi et al., 2021).

The AME procedure is typically performed by utilizing growing broilers or adult roosters that are used only once for an AME determination, therefore, potential differences in genetics and growing conditions from each independent AME determination add variability to the results (Wu et al., 2020). Studies have also shown that age affects the determined AME values of feedstuffs (Farrell, 1978; Wu et al., 2020).

Once AME values are determined, they are typically nitrogen corrected (AME_N) to a zero-nitrogen balance. This is done for comparative purposes so that all values obtained are on a nitrogen equilibrium basis, with the assumption that all nitrogen retained primarily in the form of amino acids, will eventually be excreted as uric acid during tissue turnover, and the excretion of nitrogen is energy costly (Hill & Anderson, 1958; Sibbald, 1979). Furthermore, because nitrogen retention varies based on bird type and age, nitrogen correction reduces the variability of AME values determined in different type and aged birds (Lopez & Leeson, 2008b). More importantly, it allows high protein containing feed ingredients to be accurately compared to carbohydrate and lipid-rich feed ingredients. Protein-rich feed ingredients such as soybean meals and animal byproducts, have a good level of metabolizable energy. However, when feeding such feed ingredients during the AME procedure, any nitrogen retained as amino acids by the animal will eventually be metabolized and the nitrogen excreted at an energy cost (Abdollahi et al., 2021).

Even amino acids incorporated into tissue will eventually be turned over and replaced, leading to their catabolism and nitrogen excretion (Adedokun et al., 2011). Incorporating nitrogen into waste products, such as uric acid, and excreting them costs the animal a significant amount of energy (Hill & Anderson, 1958; Sibbald, 1979). Therefore, the AME of feed ingredients should be decreased for the eventual cost of the nitrogen excretion, and this decrease in energy will be much greater for protein rich ingredients, such as soybean meal, than for low protein carbohydrate and/or lipid-rich ingredients, like corn (Dale & Fuller, 1984; Abdollahi et al., 2021).

TME_N determination

In the TME procedure, adult single comb White Leghorn roosters are typically used as they are no longer growing and are not producing eggs, which reduces the variability between birds (Guillaume & Summers, 1970). The roosters undergo a 24-hour feed withdrawal to clear their digestive tracts (Sibbald, 1979) and are then precision fed a fixed amount, usually 30 to 35 grams, of a pure feed ingredient. In this single feeding method, the roosters are not fed more than 35 grams to prevent crop impaction (Sibbald, 1977; Sibbald & Morse, 1983). Typically, between 8 and 10 roosters are fed per ingredient and an additional 8 to 10 roosters that were fasted for 24 hours will not be fed (Dale & Fuller, 1984). Excreta is then collected for the following 48 hours from both the fed and unfed roosters (Dale & Fuller, 1984). The feces collected from the unfed roosters serve as the endogenous control, and the gross energy content of the endogenous feces will be used in correcting the gross energy content of the excreta of the fed birds (Sibbald & Morse, 1983).

In the TME procedure, because the roosters are fasted for 24 hours before the test ingredient is fed, they are in a negative nitrogen balance at the start of the procedure. When the

nitrogen correction procedure for TME was developed, Dale and Fuller (1984) reported that the roosters remained in a negative nitrogen balance throughout the procedure. However, the degree of the nitrogen deficit decreased as the nitrogen content of the ingredients increased (Dale & Fuller, 1984). However, to avoid overestimation of endogenous energy correction, a nitrogen correction was applied to account for differences between the energy voided by fasted roosters and the endogenous portion of the excreta energy of roosters receiving purified carbohydrates (Dale & Fuller, 1984).

Since its conception, the TME bioassay is more advantageous than the AME procedure because the birds are precision fed a known amount of a single test ingredient without any feed refusal (Sibbald, 1976). There is no need to feed a complete diet with a portion of it being a test ingredient like in the AME bioassay. The AME procedure is prone to errors with spilled/wasted feed and feed contamination of the feces being collected (Sibbald, 1985). For the TME_N procedure, there is also not a need for an acclimation period as required in the AME procedure as the birds are precision fed the test ingredient. The AME procedure is completed using young, growing birds, which typically precludes them from being used again for further AME testing (Leeson & Summers, 2001). However, a colony of adult roosters can be used multiple times in a given year to test many different feed ingredients for TME_N determinations, and their growth, metabolism, genetics, and space/facility requirements are constant, unlike the test birds used in AME_N determinations (Sibbald, 1976), which makes the use of roosters favored for the TME_N procedure. Determined TME_N values of ingredients, such as corn, soybean meal, and poultry byproduct meal have shown to be similar among roosters and broilers (Dale & Fuller, 1980). Due to the similarity between TME_N and AME_N values (Baidoo et al., 1991), TME_N has become a popular choice by nutritionists to be used in feed formulations.

The TME_N procedure does have some negative aspects. The amount of endogenous loss actually varies relative to the amount and physical characteristics of the feed ingredient passing through the gastrointestinal tract (Farrell, 1981; Tenesaca & Sell, 1981; Hätel, 1986; Farrell et al., 1991). However, in the TME_N procedure the endogenous waste is constant as it is determined from unfed birds. However, it is important to remember that the AME_N procedure does not even factor in a correction for endogenous waste. Another criticism is that, when one feed ingredient is fed alone in the TME_N procedure, it eliminates the possibility of synergistic or antagonistic effects between multiple ingredients found in a complete diet. For example, the increased presence of fat in a diet can add to the energy derived from other ingredients (Young, 1961; Artman, 1964).

Limitations of energy evaluation bioassays

Although cheaper than the NE determinations, the AME and TME bioassays are still very time consuming and expensive, with a cost typically between 700 and 1,500 dollars per ingredient. In addition to the time to complete the animal bioassays, subsequent laboratory analyses of the test diets or ingredient as well as the collected excreta often take over two weeks to complete. Considering the lack of ingredient storage capacity and the tonnage of completed diets that a feed mill produces in a week, any ingredient tested using the AME_N and TME_N bioassays will have already been fed before the results are available.

Because of the cost and time needed for the AME_N and TME_N bioassays, researchers have developed regression equations that are based on rapid proximate analysis of a feed ingredient sample to estimate AME_N and TME_N. Feed ingredients that these equations have been developed for include dried distiller's grain with solubles, bakery meal, peanut meal, poultry by-product meal, canola meal, and corn gluten meal (Pesti et al., 1986; Dale et al., 1990; Dale, 1996; Batal

& Dale, 2006; Latifi et al., 2023). However, these equations were typically based on a limited number of samples that often did not reflect the wide range of AME_N and TME_N values obtained in a given feed ingredient due to processing and/or agronomic differences, and thus these equations need to be validated with more diverse sample sets (Meloche et al., 2013; Meloche et al., 2014; Latifi et al., 2023). In addition, even though these prediction equations often have R^2 values of 0.90 or greater, the values predicted by these equations can easily be plus or minus 10 to 20 percent from the animal bioassay determined values (Dale et al., 1990; Meloche et al., 2014; Latifi et al., 2023). These equations also express ME on a dry matter basis. This further diminishes the functionality of these equations as nutritionists formulate on an "as is" basis, as that is how the ingredients are received at each feed mill (Batal et al., 2005; Meloche et al., 2014; Latifi et al., 2023). Moisture levels fluctuate between ingredients as well as lots of the same ingredient (Tabib et al., 1981; Islam et al., 2015). Thus, to have accurate specifications, moisture levels would need to be determined. Thus, there is a need for a more rapid way to determine ME on "as is" basis which will allow nutritionists to accurately formulate diets in real time to meet the energy requirements of the birds in the field using determined ME values obtained as each shipment of the ingredient arrives at the feed mill.

Summary

Dietary energy is an important component of poultry diets as it fuels the bird's daily maintenance needs as well as provides the energy needed for growth and reproduction. The goal of a commercial poultry nutritionist is to meet dietary requirements of the bird while also using the most cost-efficient diet formulations. When formulating poultry diets, a nutritionist would ideally have either the AME_N or TME_N values of their ingredients. However, the animal bioassays to determine these values are costly and take weeks to complete. Thus, the nutritionist

is forced to use reference values for such ingredients even though these reference values are simply an average of determined values and may not reflect the wide variation seen in AME_N or TME_N values for an ingredient based on processing and /or agronomic differences. Therefore, the use of these reference values can lead to the formulation of diets that have an excess or deficiency of energy.

CHAPTER 2

NEAR INFRARED REFLECTANCE SPECTROSCOPY

Near-infrared reflectance spectroscopy (NIRS) is a non-destructive analytical technique that has the potential to revolutionize the way the nutrient profiles of feed ingredients are obtained. The NIRS procedure provides rapid results, does not use harsh chemicals, and produces no hazardous chemical waste. Once calibrated, an NIRS machine can determine the concentration of multiple nutrients from a single scan of a feed ingredient rather than individually testing for each nutrient. The NIRS analysis involves the selective absorption of electromagnetic radiation when a sample is scanned in the near infrared region of the electromagnetic spectrum from 730 to 2500nm (Givens et al., 1997). A spectrum of absorbance of the sample can be plotted and analyzed to determine the chemical composition of the sample. The absorbance spectra are based on vibrational frequencies of the bonds between the atoms of a molecule, and depending on the chemical elements that constitute the molecule, the molecule will have specific vibrational signatures (De Boever, 1994; T. N. Smith et al., 2001).

History of Near Infrared Reflectance Spectroscopy

The near infrared region of the electromagnetic spectrum was first discovered by William Herschel (1800) when he detected radiation past that of visible light. Over 100 years later, William W. Coblentz (1903) was the first to measure the spectra of organic fuchsine and inorganic cyanine within the near infrared region. From there, spectrometers for the NIR region were built, and this expanded the use of absorbance spectrums to determine chemical parameters of selected samples. By 1965, Karl Norris of the United States Department of Agriculture,

employed NIRS to predict the moisture content of grains and feeds (Norris, 1965). As the use of NIRS to determine the concentrations of organic materials continued, it became clear that there were only a few areas in the near infrared spectrum where the absorbance was due to only one specific chemical functional group in a compound. For example, C-H, N-H, and O-H bonds and CH_3, NH_2, and COOH functional groups are common components found in carbohydrate, lipid, and/or protein molecules and, thus, do little to distinguish these compounds directly. Therefore, it was determined that statistical tests were the key to accurately distinguishing secondary relationships between spectral data to identify and quantitate the molecule of interest from similar molecules in a sample, while correlating this information with the laboratory determined concentration of the molecule of interest (Foley, 1998; Fearn, 2005). As the advancement of computing power and statistical analyses has continued, the capability of NIRS to accurately predict the content of a molecule of interest in a sample has increased greatly.

With NIRS, because the molecular bonds of the compound of interest are absorbing specific wavelengths of infrared light in a signature manner when there is more of the compound more infrared light will be absorbed and less will be reflected back to the detector of the NIR instrument which actually measures the proportion of light which is reflected by the sample. Photosensitive materials such as lead, sulfide, or silicon on the detector converts the reflected light into electrical signals that are recorded to create an absorption spectrum that is analyzed by the instrument's computer software program to quantitate the compound of interest. Because NIRS analysis is dependent on detecting reflected light, light scattering must be minimized when a sample is scanned in the NIR spectrum. Scattering can occur when the reflected light is re-reflected, refracted, or diffracted by the contents of the sample being analyzed. Light scattering is

most affected by the particle size of the sample, its moisture content, and its temperature. Therefore, uniform sample preparation for NIRS is crucial.

NIRS sample preparation

Grinding. Proper sample preparation can have substantial effects on both the spectral data and corresponding calibration results. First, a sample that is representative of the entire batch of ingredient should be collected. The sample should then be ground to a particle size that ensures the NIRS light can penetrate the sample. Consistency of the technique to grind particles down to a homogenous size that does not create spectral interference is key, as NIRS is responsive to both the chemical and physical characteristics of the sample (Murray & Cowe, 2004). A uniformly ground sample increases the overall accuracy of NIRS prediction calibrations (Fontaine et al., 2001). Mean particle size and distribution have been shown to affect reflectance properties and can even account for up to 90% of spectral variance (Givens et al., 1997). In addition, it is important that the grinder chosen to prepare feed ingredient samples has the capacity to dissipate heat quickly and not alter the moisture content of the feed ingredient being ground. Heating of the sample during grinding could alter the chemical nature and, thus, nutrient availability through heat stimulated nonenzymatic reactions such as the Maillard reaction or denaturation of compounds (Hurrell and Carpenter, 1974; Leiva et al., 2022). Because feed ingredients are utilized in diets on an “as is” basis, if water evaporates during grinding, the concentration of the remaining nutrients in the diets will be increased because of the loss of water. Thus, the NIRS determined nutrient concentrations on a sample that lost significant moisture during grinding would not be directly reflective of the content in the ingredient being incorporated into a diet.

The two methods commonly used for feed grinding utilize either shearing or impacting force to decrease the particle size of a sample. Shearing mills, such as the Wiley mill, reduce the particle size with the use of knives before the sample is forced through a sieve into the collection vessel, whereas impaction grinders decrease the particle size of a sample by crushing the feed against a silicon carbide-impregnated wall before it passes through a sieve. Bakalli et al. (2000), reported that there were significant differences in analysis outcome for whole soybeans ground with a Cyclotec (impact) versus a Wiley (shearing) grinder. It was also found that even though a Cyclotec grinder caused a 3% loss in sample moisture due to more air exposure, preparing samples with this instrument led to less experimental error (Bakalli et al., 2000). Different sized sieves or screens are utilized throughout literature. There is roughly equal representation of 1mm screens (Valdes et al., 1985; Van Kempen & Bodin, 1998; Bakalli et al., 2000) and 0.5 mm screens (Bakalli et al., 2000; T. N. Smith et al., 2001; Fontaine et al., 2002) being reported in the literature. Because an NIR spectrometer is very sensitive to the amount of sample surface presented for analysis, it is imperative that the same size screen, regardless of size, is used for all samples to build a calibration model and the subsequent samples using the calibration model for analysis (Foley, 1998).

Temperature. Before use, the near infrared reflectance spectrometer should be allowed at least 15 minutes, and may require as much as an hour, of warm-up time depending on the manufacturer's instructions for the NIRS analyzer. The spectral analysis of samples is very sensitive to the temperature of the machine and, as the lamp and detector warm up, there is a temperature gradient that occurs within the instrument that will lead to inconsistencies in the spectral readings if samples are scanned during the warm-up period. Samples should also be at room temperature prior to scanning (Westerhaus, 1989a). Samples should be allowed to rest and

dissipate heat after grinding to mitigate temperature variation. This also applies to samples that have been stored in a freezer as they need reach room temp prior to scanning. A difference in sample temperature will affect the vibrational frequencies of the bonded organic elements within the sample, which can cause changes in the spectral absorbance bands (Hansen et al., 2000).

Moisture. Excess moisture in a sample can also skew NIRS results. Water is a strong absorber in the NIR region, and this means that high moisture sample spectra are dominated by the signature of water (Büning-Pfaue, 2003). In addition, water molecules naturally reflect and refract light, which increase light scattering for NIRS detection. Several studies have shown that excessive moisture in samples decreases the R^2 and increases standard error values of NIRS calibration curves when compared to calibration curves developed using drier samples (Abrams et al., 1988; Reeves III et al., 1989; Reeves, 1995; Büning-Pfaue, 2003). Excessive moisture in a sample causes O-H bond stretching and O-H deformation bond vibrations in the 1850-2000 nm region of the spectra which can cause detector saturation and, thus, analysis distortion (Baker et al., 1994; Givens et al., 1997). The presence of excessive moisture and associated detector saturation in a given sample spectra can cause error when predicting the concentration of nutrients (Givens et al., 1997).

Samples prepared for NIRS can be stored in a freezer in glass bottles (Valdes & Leeson, 1992), polyethylene bottles (Fontaine et al., 2002), or plastic bags (T. Smith et al., 2001; Tahir et al., 2012) to minimize oxidation or other chemical changes during storage so they can be utilized again for NIRS analysis, if necessary.

Preprocessing of spectra

Even with meticulous and consistent sample preparation, it is sometimes necessary to use spectral preprocessing methods to optimize spectral output. Spectral preprocessing methods are

used to treat individual sample spectra to reduce background noise which therefore increases the chemical information of the spectra in order to properly develop a calibration curve (Shi & Yu, 2017). Spectra effects that warrant pretreatments include the overlapping of absorbance bands, non-linearity, and light scattering (Shi & Yu, 2017; Chou, 2021). Multiplicative scatter correction is a common pretreatment method used to reduce distortions of the spectra due to light scattering by removing these spectral distortions from the reflectance output generated by the molecular bonds of the sample scanned. First and second derivative preprocessing are used to correct for overlapping absorbance bands. The first derivative method emphasizes spectra signals that have steep edges rather than flatter bands. By calculating this derivative, detected spectral output bands get a steeper edge thus making their evaluation easier (Conzen, 2014). However, when calculating this derivative, spectral noise may also be enhanced which can distort the chemical signal of the sample. In comparison to first derivative, with calculation of second derivative preprocessing, even relatively flat spectral output can be evaluated. However, by doing this, the spectral noise is further enhanced, thus this method is only applied to a sample's spectral output from narrow ranges within the NIR spectrum which has minimal spectral noise (Conzen, 2014). There is no general rule for choosing which preprocessing method or methods to use on NIRS spectral output from a sample, as it is usually a trial by error method with most measurements benefiting from preprocessing. However, in some cases spectral output is not further improved by employing pretreatments, and preprocessing can actually be detrimental (Esteve Agelet & Hurburgh, 2010).

Analysis of data and development of a calibration

Once spectral data is obtained for a group of samples, the goal is to establish a calibration curve that relates the spectral wavelength scans of the group of samples, which have been

scanned in the NIR spectrum to the known concentration of the molecule of interest in these samples determined through chemical or biological analyses of these samples. For example, in feed analysis, the molecule of interest could be the amino acid lysine. The created calibration curve for lysine can then be used to predict the concentration of lysine in future samples. Modern NIRS systems are equipped with powerful analytical software that searches the spectral scans of the samples for unique and definitive signatures that specifically correlate with the provided known concentration of the molecule of interest in the samples.

In order to make this correlation between the spectral data of the samples and the known concentration of a molecule of interest, NIRS relies on chemometrics, which is defined as "the discipline that uses mathematical, statistical, and other methods to design or select optimal measurement procedures and experiments, and to provide maximum relevant chemical information by analyzing chemical data and provides the ability to process large quantities of data into useful methods of prediction and analysis" (Deming et al., 1988; Kumar et al., 2014). Chemometric tools used in NIRS include quantitative calibration and validation methods, wavelength selection, and the selection of principal components. Chemometrics uses multivariate regression methods to distinguish relationships between the samples in order to find a statistical correlation between the spectral data and the known reference value of the samples used for the calibration (Fearn, 2005; Heberger, 2008; Kumar et al., 2014).

Three of the best known multivariate chemometric methods that are available to be used for developing quantitative NIRS calibrations are multiple linear regression, principal component regression, and partial least squares regression. However, principal component regression and partial least squares regression are the two that are most used (Feinberg, 1990; Phatak & De Jong, 1997; Chou, 2021).

Multiple linear regression was first developed by Sternberg et al (1960), and it was the method that was used by Norris in his initial work in NIRS. Using multiple linear regression in NIRS results in the selection of a few spectral outputs from only wavelengths in the NIR spectrum that display the strongest correlation between sample absorbance spectra and determined reference values (Pérez-Marín et al., 2007). One potential downfall with this method is that multiple linear regression does not consider wavelength multicollinearity or variable codependency, which is important in complex samples such as feed ingredients where physical and chemical properties are codependent and complex molecules are bound together and influence the absorbance across different wavelengths (Esteve Agelet & Hurburgh, 2010). Thus, the selection of only a few wavelengths to provide analysis makes the calibration the least reliable of the three methods, as reviewed by Esteve Agelet and Hurburgh (2010).

Principal component regression combines principal component analysis and multiple linear regression. Principal component analysis first splits up spectra according to their spectral patterns using the full spectral range (Devaux et al., 1988; Naes & Martens, 1988; Chou, 2021). Then multiple linear regression is implemented to further extract correlations of the spectral outputs using selected wavelengths. Even though principal component regression is a further refinement for calibration, unnecessary data may end up being used to generate correlations due to the entire spectral range being used for principal component analysis (Naes, 1987; Naes & Martens, 1988; Chou, 2021).

Partial least squares was introduced as an improved alternative to principal component regression, and it has become the preferred method for a few reasons (Wold, 1975; Esteve Agelet & Hurburgh, 2010). Partial least squares performs multiple replications to determine the factors or primary components for the calibration model using both the spectral data and reference

values (Esteve Agelet & Hurburgh, 2010; Conzen, 2014). By using both the spectral data and reference values as part of the calculations, unwanted factors are not used, and instead only the factors that have the highest correlation between reference values and spectral data are utilized. This leads to a more precise calibration which will then permit the prediction of unknown samples (Conzen, 2014).

The importance of choosing an adequate calibration sample set is usually understated. Windham et al. (1989) recommended that a minimum of 50 samples be used for an initial calibration and that 10 to 20% of the total population of samples be used for the calibration in larger data sets (Westerhaus, 1989b). For an ideal and accurate calibration, chosen samples must mirror the variances that will be encountered in the physical form of the samples and concentration range expected for the parameter of interest in samples to be tested later using this calibration curve (Fearn, 2005). Sources of variance in nutrient concentrations for poultry feed ingredients could come from differences in variety, location, crop year, as well as external processing and storage differences. If these sources of variability are not accounted for in the calibration samples, the resulting curve will not be as suitable for predicting a compound of interest in subsequent samples having these variables (Fearn, 2005). The accuracy of the determined bioassay or chemical reference values for the parameter of interest are paramount because NIRS determinations are calibrated to mimic the reference method, and thus inherit the variability of error associated with the reference method.

Once calibration samples have been chosen and the calibration made, the calibration model must be optimized. The calibration will be presented as a regression model curve of the true values versus the calibration predicted values. Outliers from either reference values or from spectra data may exist, and calibration curves are very sensitive to them (Kovalenko et al., 2006).

If the calibration curve has visible sample outliers, the samples' spectra and reference values should be analyzed to ensure that there was not a determination, preparation, or scanning issue. In some cases, outlier samples are excluded from the calibration curve if the spectral and wet chemistry values indicate the sample may have had atypical or poor qualities such as degradation (Van Kempen & Bodin, 1998). In addition, based on examination of the regression model of the calibration, samples can be added to improve the curve or deleted if the samples are providing duplicate contributions to the curve.

Validation of the model

Once the calibration curve model is created, it must then be validated. A suitable validation is crucial to determine if the calibration curve is appropriate for the prediction of the content of the compound of interest in new samples, which is the goal in creating a NIR calibration. For the validation, a test set of samples, which have had the content of the parameter of interest determined by bioassay and/or chemical analyses, will have the concentration of this compound predicted by an NIRS calibration curve. The laboratory determined and NIRS predicted values are then compared to one another, typically by regression analysis, to determine the robustness of the calibration curve in predicting the concentration of the compound of interest. Again, it is important that the physical characteristics of the validation samples and the concentration of the parameter of interest in the validation samples be reflected in the samples used to build the calibration curve.

The two methods used in the validation step are the cross-validation method and the test set method. The cross-validation method is best used when analyzing a smaller test set, usually less than 50 samples (Conzen, 2014). This method runs a validation in which one sample is taken from the calibration set and validated using the remaining samples in the calibration. A

comparison of prediction results to the reference values shows how accurate the model predicts. The sample that was analyzed is returned to the calibration, and a new sample is removed until the entire data set has been validated.

The test set method is used when analyzing a data set larger than 50 samples. This is the ideal method, as it is accomplished using samples that were not previously used for the calibration model. This method is usually accomplished by having two-thirds of the samples in the calibration set and one-third of the samples in the validation set. However, the number of the samples in the validation test set can be increased beyond the one-third recommendation if they are available. All samples should be independent of one another, and no replicates of samples should be used in both the calibration or validation sample set (Conzen, 2014).

NIRS Statistics

Calibration and validation statistics are calculated and used to illustrate the robustness of each curve. Calibration and validation curves have their initial robustness evaluated by the coefficient of determination (R^2) and the residual predictive deviation (RPD). The R^2 illustrates how close the data are fitted to the regression line. It explains the variance between the predicted and reference values and how well they fit the model (Fearn, 2002). A closer value to 1 indicates a better correlation between the laboratory determined reference values and the NIRS predicted values. However, the R^2 value has become somewhat irrelevant when it is used to describe the appropriateness of a model as the value is heavily dependent on the range of values used (Davies & Fearn, 2006). The RPD value is related to the model's ability to predict future unknown samples, as it relates to the variability of the calibration data. RPD values of 3 or above normally indicate that the calibration is useful in predicting the concentration of the compound of interest (Williams, 2001).

Another statistic called the standard error of the estimate is calculated for the calibration curve. The standard error of the estimate represents the analysis error of the calibration, with lower values being indicative of a better fit (Conzen, 2014). For the validation curve, the standard error of prediction is the equivalent to the standard error of the estimate, and it is a measure of the variability of the difference between the predicted and reference values. It is an estimate of what the difference will be when the calibration is used for future predictions of unknown samples (Davies & Fearn, 2006). For simplicity, it is calculated by using the root mean square of differences, with a lower value being indicative of a better fit.

For feed ingredient analyses a more practical evaluation of the validation data set is often completed in which the percent deviation of the NIRS predicted value from the laboratory determined value is calculated (Cope, 2021). The previously described statistical evaluations, such as R^2 and RPD, focus on an overall evaluation rather than on an individual sample basis. While the overall statistics are important, when formulating diets to meet dietary requirements, a nutritionist needs to know how large the deviations from the actual values can be so that they can formulate diets with a safety margin to compensate for any potential underestimation of the content of the nutrient in the feed ingredient determined by NIRS.

NIRS in feed ingredients and diet formulation

The proximate composition of feed ingredients, including gross energy has been determined using NIRS (E. Finney et al., 1978; Valdes & Leeson, 1994; T. N. Smith et al., 2001). In addition, NIRS has proved to be accurate in directly predicting levels of compounds within certain feedstuffs, such as phytate-phosphorus and antinutritive factors like gossypol (Ben-Gera, 1968; T. N. Smith et al., 2001; Lordelo et al., 2008; Aureli et al., 2017). While the use of NIRS has been well documented in measuring specific nutrient components, its use to

predict the digestibility of nutrient components has not been established in published research. The ME determination of ingredients by NIRS is typically based on using longstanding equations to predict ME based on proximate analysis reference values, which can also be determined by NIRS. The accuracy of ME values using actual proximate analysis results has never been greater than about 90%, and this accuracy is decreased further once the error associated with NIRS determinations of proximate values is added into the prediction (Mateos et al., 2019). Thus, there is a need to develop NIRS calibration and validation curves for AME and TME to avoid the use of inaccurate estimations of these values. However, the expense of the AME and TME bioassays (Valdes & Leeson, 1992; Bastianelli, 2013) has made such undertaking slow to occur (Yegani & Korver, 2012).

Nutritionists formulate diets using conservative nutrient values of feed ingredients to mitigate any potential for formulating diets with nutrient deficiencies (Van Kempen & Simmins, 1997). This is especially true in broiler feed formulation as broilers are fed between three and five diets throughout their production cycle, which typically runs from day of hatch to 35-56 days of age (Moss et al., 2021). Using NIRS ingredient analyses has the potential to allow nutritionists to obtain real time nutrient specifications of their ingredients and aid in the formulation of diets to reduce over formulation (Van Kempen & Simmins, 1997) and to minimize nutrient deficiencies. As previously discussed, the use of animal bioassays and wet chemistry to obtain metabolizable energy values are expensive, laborious, and take several weeks to complete. The use of NIRS has the potential to allow feed mills to receive quick results on every batch of an ingredient that arrives which will aid nutritionists to formulate diets based on determined values. The ultimate goal of feed analysis is to maximize ingredient value (Bastianelli, 2013). Using NIRS as a quality control measure for incoming trucks or rail cars has

already been shown to reduce feed cost and increase return on investment (Ramaswamy et al., 2016).

However, the use of NIRS in feed manufacturing does have impediments. One disadvantage associated with the use of an NIRS machine is the successful transfer of calibrations from one machine to another (Yang et al., 2019). Differences in machine brands as well as laboratory conditions and grinding methods can make a transferred calibration unusable (Givens et al., 1997). In most settings, the calibration curves from one machine are electronically transferred to another only if the two machines are manufactured from the same company. A few select feed samples from the original validation set are then used to validate the transferred calibration to ensure the accurate transfer of the calibration. If this does not work or if transferring a calibration between machines with different manufacturers, all stored samples that were used for the original development of the calibration curve and validation test set must be scanned and assigned reference values to create a new calibration and validation for the new system. This is very laborious and not very practical, if the calibration needs to be transferred to many different NIRS systems, due to the large numbers of frozen samples that need to be transferred and scanned to recreate the calibration. Another disadvantage is the entry cost of an NIRS benchtop machine which is over $100,000 (Brown et al., 2020). This high initial cost as well as the cost associated with hiring an NIRS technician to operate the system has deterred many from fully embracing the technology. However, there are commercially available portable NIRS devices. These portable or handheld devices are transportable and less costly than benchtop models with some having an associated cost under $5,000 (Pu, et al., 2021). However, these portable NIRS have a limited spectral range and are equipped with a limited number of preprocessing techniques for spectral data, and thus must be used in tandem with a benchtop

model to develop the robust calibration models to be transferred to the handheld models used in field situations (Pu et al., 2021; Rukundo et al., 2021).

Summary

In summary, NIRS is a rapid analysis method that enables a Multi-Purpose Analyzer to be calibrated to predict nutritional component concentration values after analyzing the reflectance of the feed ingredient in the near infrared spectrum. Once a NIRS calibration has been developed and validated, determinations of the parameter of interest in subsequent samples is fast, involves no chemical, and creates no waste. Near infrared reflectance spectroscopy is an analysis method with a wide array of uses in the feed industry. While NIRS has a well-established capability to measure specific chemical components in feed ingredients or mixed feeds, its utilization in measuring the availability of a nutrient component in feed ingredients or feeds has not been investigated until recently.

CHAPTER 3

STATEMENT OF PURPOSE

Accurate poultry diet formulation depends on knowing the actual nutritional values of the feed ingredients utilized in making the diet. Feeding poultry improperly balanced diets leads to poor animal production, increased environmental pollution, and economic inefficiency, given that feed accounts for roughly 70% of live poultry production costs. When evaluating feed ingredients for poultry diets, one of the most important nutrient specifications to have is the nitrogen corrected true metabolizable energy (TME_N). This is because it is the available energy that is going to sustain and fuel the bird's rapid growth from hatch to market weight in the broiler industry and egg production in the breeder and table egg markets. A rooster bioassay is used to determine TME_N of feed ingredients for poultry diets. This bioassay is costly, and it takes one to two weeks before results are available. Thus, in commercial feed mill settings, the results cannot be completed on delivered ingredients before they are utilized in diets and fed to poultry.

Over the past few years there has been an evolution in feeding commercial poultry in the United States. The two primary ingredients used in commercial poultry diets in the United States have been corn and soybean meal. The growth of ethanol production as an alternative to fossil fuels has led to an increase in the cost of poultry feed ingredients, especially corn. Because the digestibility of the amino acids making up the protein content of soybean meal is very high, it has become a staple in processed food production for humans, and this increased demand for soybean meal has increased its cost. Because corn and soybean meal are nutrient dense and highly digestible, this creates issues for feeding broiler breeders where the level of feed

restriction continues to increase to prevent these birds from growing too quickly and becoming reproductively compromised, as the genetics for rapid growth in broilers continues to increase. This rapid growth is good for the broiler progeny being marketed but not for their breeder parents. Thus, in broiler breeder diets the corn and soybean meal needs to be diluted with low energy ingredients that have increased fiber levels.

A few consumer-driven trends are also influencing poultry diet formulations. One of these is the avoidance of animal by-products in poultry diets (all-vegetable diet). Similarly, the production of free-range poultry, organic poultry, or poultry fed diets that are antibiotic/pharmaceutical free have all increased. Finally, poultry production companies are currently much more sensitive to providing diets that do not have nutrient excesses, as these excesses can lead to fat accretion that is carcass waste or can increase nitrogen and phosphorus excretion that can cause environmental concerns.

With these different pressures to find either cheaper or low energy feed ingredients, to utilize organically produced feed ingredients, or to use feed ingredients that may have nutraceutical properties, new feed ingredients for poultry production are being investigated and utilized. While these alternative feed ingredients are being introduced, there is also demand to accurately define the nutrient specifications of traditional ingredients to minimize the production of diets with nutrient deficiencies or excesses. The metabolizable energy content of traditional poultry dietary ingredients, like corn and soybean meal, vary based on such factors as cultivar genetics, growing conditions, and processing and storage procedures.

Near Infrared Reflectance Spectroscopy (NIRS) is a rapid analysis method that enables a Multi-Purpose Analyzer to be calibrated to predict nutrient concentrations in a feed ingredient sample after analyzing the reflectance of this sample in the near infrared spectrum. Once an

NIRS calibration has been developed and validated, determinations of the parameter of interest in subsequent samples is fast, involves no chemicals, and creates no waste. While NIRS has a well-established capability to measure specific chemical components in feed ingredients or mixed feeds, its utilization in measuring the availability of a nutrient component, such as TME_N, in feed ingredients is just beginning to be explored.

The accuracy of an NIRS calibration curve predicting the TME_N of poultry feed ingredients is heavily dependent on the accuracy of the TME_N values determined by the rooster bioassay for the NIRS calibration and validation. The TME_N bioassay for poultry was developed over 30 years ago, and thus, many currently utilized feed ingredients such as high protein isolates, low energy ingredients with high fiber content, and newer alternative feed ingredients, were not investigated. Therefore, the procedure was not validated for them. Thus, the goal of the current research was to validate the rooster TME_N bioassay using a wide array of ingredients currently used in making poultry diets, and then to use these validated values to construct an NIRS calibration curve and validation curve for the determination of TME_N in poultry feed ingredients.

CHAPTER 4

MATERIALS AND METHODS

Experiment 1- Nitrogen correction of TME

The apparent metabolizable energy (AME) and true metabolizable energy (TME) assays of poultry feed ingredients have traditionally been nitrogen (N) corrected to yield AME_N or TME_N. By correcting to zero N retention in the AME_N bioassay, all feed ingredient results are directly comparable to one another. Roosters for the TME_N bioassay are fasted for 24 to 30 hours prior to precision feeding of the test ingredient which places them in a negative N balance. Thus, the N correction for the TME_N assay was actually utilized to avoid an overestimation of the endogenous energy correction. In the original published research conducted nearly 40 years ago, roosters remained in a negative N balance when fed test ingredients and the N correction resulted in TME_N values that were 1 to 14% lower than their corresponding TME values (Dale & Fuller, 1984). However, this original research only included 13 different individual poultry feed ingredients with the TME_N determined on a total of 24 feed ingredient samples and did not include any low energy high fiber ingredients, such as rice hulls and wheat middlings, or high protein isolates that are often presently utilized in poultry diets. Therefore, in the current research, N retention, TME, and TME_N were determined on over 750 ingredient samples (Table 4.1) across more than 65 different ingredient types to determine if the original findings on N retention and correction were still appropriate.

Table 4.1. Feed ingredient samples used in the evaluation of nitrogen corrected true metabolizable energy values and near infrared reflectance spectroscopy (Experiments 1-7).

Ingredient type	Sample count	Ingredient type	Sample count
Algae	5	Meat and bone meal	125
Almond shells	5	Peanut meal	9
Bakery Meal[1]	67	Pennycress meal	5
Black soldier fly larvae	6	Poultry by-product meal	31
Canola meal	18	Poultry tankage	26
Corn	63	Sorghum	9
Corn germ meal	8	Soybean hulls	15
Dried distiller's grain	10	Soybean meal	73
Dried distiller's grain with solubles	72	Supermarket waste by-product meal	6
High protein dried distiller's grain with solubles	17	Wheat	5
Feather Meal	16	Wheat middlings	14
Fermented products[2]	12	Other ingredients[3]	
Isolated soy protein	25		

[1]Bakery meals included conventional bakery meals, all vegetable bakery meals, and ingredient specific cookie and cereal bakery meals

[2]Fermented soybean meals, fermented DDGS, and fermented corn

[3]Ingredients with fewer than 5 total samples and they include: amino acid manufacturing by-product, alfalfa meal, arrow root leaves, blood meal, Brazil nut powder, barley, calliandra leaves, carinata meal, cassava leaves, corn gluten feed, corn gluten meal, cottonseed meal, cricket larva meal, adult cricket meal, dried grape pomace, dried waste water, flaxseed meal, isolated fiber component of DDGS, hatchery waste meal, hominy, high protein rice product, hempseed presscake, hempseed leaves by-product meal, hempseed powder, jatropha meal, moringa powder, oats, palm kernel cake meal, pea meal, pongamia meal, rice bran, rice hulls, rice mill feed, rye meal, experimental fish meal/soybean meal blend, spinach powder extract, sugarbeet root powder, sunflower meal, sweet potato leaves, single cell product, yeast cells, poultry blood waste meal.

TME_N bioassay of feed ingredients

All procedures involving live birds were approved by the University of Georgia Animal Care and Use Committee. Each year, 400 day-of-hatch Hy-Line W36 Single Comb White Leghorn male chicks were obtained from the Hy-Line North America LLC, Mansfield, GA Hatchery. The birds are raised in a room that measured 7.31 by 6.14 meters, that contained four plastic pan feeders (0.14 $meters^2$) and two water lines, each equipped with 12 nipples. A single environmental controller for the room regulated a single natural gas-fired furnace, one 46-centimeter ceiling circulation fan and two exhaust fans, one measuring 53 centimeters and the other one measuring 26 centimeters. Inlet air was brought in from a hallway equipped with a separate environmental controller regulating two gas fired furnaces, two 46-centimeter ceiling circulation fans, and an evaporative cooling system. Ambient room temperature was set at 34°C on day 0 of chick age and decreased by 0.28°C per day until 24°C was reached and maintained through 20 weeks of age. The light intensity was 20 lux from day 0 to 14 days of age. At 2 weeks of age, the intensity was reduced to 2 lux and was maintained at this level to 20 weeks of age. The cockerels received 23.5 hours of light on day 0 of age, and this was reduced by 30 minutes each day until 10 hours of light per day was reached and maintained until 20 weeks of age. The birds were given ad libitum access to feed and water throughout this period; all diets were formulated to meet or exceed NRC guidelines (1994).

At 20 weeks of age, roosters were relocated to a single room containing six, 64 individual cage battery units with cages measuring 35.5 centimeters wide, 45.7 centimeters deep, and 61.0 centimeters tall. Each cage was equipped with a single nipple drinker and had access to a feed trough. The room measured 12.4 meters wide by 14.5 meters long and was ventilated by a positive pressure ventilation system. The system provided central heating and cooling via a

natural gas fired furnace and an integrated evaporative cooling system. The temperature and ventilation were controlled by a single environmental controller. There were also 5 circulation fans measuring 66 centimeters in diameter controlled by an independent timer, set to circulate air for a minimum of one minute out of each five-minute time cycle. Lighting intensity was maintained at 20 lux, and light was provided for 16 hours per day. The birds had free access to water and a nutritionally complete diet when not being utilized for TME_N determinations.

The TME_N bioassay that was utilized followed the method of Dale and Fuller (1984) with slight modifications. In brief, adult roosters, between the age of 22 and 80 weeks, were utilized from the battery cages. For each feed ingredient tested, 8 to 10 roosters were feed fasted for 30 hours prior to feeding to empty their digestive tract. Fasted birds were then transferred to individual suspended wire cages that measure 30.5 centimeters wide, 45.7 centimeters long (depth), and 50.8 centimeters tall. Each cage was equipped with a nipple drinker to provide free access to water and a stainless-steel feces collection pan. Each rooster was precision fed 35 grams of the test ingredient. If the ingredient was low in density, such as rice or soy hulls, less than 35 grams was fed to prevent crop impaction with a total volume being fed never exceeding 100 milliliters. After precision feeding, the bird was placed in its collection cage, and excreta was collected from each individual rooster for the following 42 hours. An additional 10 un-fed roosters, which served as the control for endogenous losses, had their feces collected for the 42-hour collection period as well. The fed and endogenous control roosters did not have access to feed throughout the duration of their 42-hour feces collection period. At the end of the feces collection period, the birds were returned to their original daily living cages. The excreta from each rooster was scraped from the stainless-steel pans and dried at 60°C for 18-30 hours (Dale et al., 1985). After the samples were dry, they were individually weighed. Weighed samples were

then combined from the 8-10 roosters to create two replicate excreta samples for each feed ingredient that was fed. The individual replicate fecal samples were ground using a KitchenAid® (KitchenAid®, Benton Harbor, MI) coffee grinder, sifted through a 1-millimeter mesh sieve, and placed into labeled sample bags for subsequent analyses. Individual roosters were not utilized for the TME_N determination procedure more than once within a three-week period. Once the roosters reached 80 weeks of age, they were euthanized and replaced in the battery cages by a new flock of 20-week-old roosters.

Chemical reference method TME_N

Gross energy and total nitrogen were determined for all feed ingredients fed to the roosters and for the dried excreta from both the fed and endogenous control roosters. These analyses were completed by the University of Georgia Agricultural and Environmental Services Laboratory (Athens, GA). A Parr 6400 Calorimeter (Moline, IL) was used for gross energy determination following the ASTM (2017) and ISO (2009) methods. Total nitrogen content for each sample was determined using the Dumas method (AOAC, 2012).

TME_N calculation

Once the gross energy (kcal/kg) and nitrogen (%) content of both the feed ingredient and corresponding feces are determined, the values were then utilized to calculate the TME_N of the test ingredient using the following equation:

{gross energy of the feed on a gram basis multiplied by the grams fed – [(gross energy of the excreta on a gram basis multiplied by the excreta dry weight in grams) – 8.73 multiplied by the (grams of dry excreta multiplied by the nitrogen content of the excreta) – (grams of the feed multiplied by the nitrogen content in the feed)] – [(grams of endogenous control dry excreta multiplied by the endogenous control excreta gross

energy on a gram basis) – (grams of endogenous control dry excreta multiplied by the endogenous control nitrogen multiplied by 8.73)]}. This is all divided by the grams of feed fed and then this result is multiplied by 1000 to go from kilocalories energy per gram of diet to kilocalories per kilogram of diet.

The factor of 8.73 reflects the correction factor for voided nitrogen that is comprised of mostly uric acid (Titus et al., 1959).

Experiment 2- NIRS all poultry feed ingredients

After obtaining TME_N values for the feed ingredients in Experiment 1, research was conducted to improve the NIRS calibration and validation curves for the determination of TME_N in all poultry feed ingredients developed previously by Reid (2017) and Cope (2021) . For this research a Bruker MPA: FT-NIR Spectrometer (Bruker Optics, Billerica, MA), outfitted with sample rotator to ensure uniform mixing of the sample while it is scanned, was used to perform the NIRS analysis of each sample. A background scan was completed prior to each ingredient scan. The MPA system included OPUS Version 8.5 software (Billerica, MA), which was used for constructing the calibration curves and for the validation of each curve. Before each use, the Bruker MPA was allowed to warm-up for a minimum of 30 minutes, as recommended by the manufacturer.

Samples used for the calibration and validation curves were selected from the same pool of samples, but the samples used in the calibration test set were independent from those used in the validation test set. Although full spectral scans were completed on each ingredient, optimization software that was included with OPUS determined the most appropriate wavelength regions to analyze and applied the optimal data pre-processing treatments to each calibration and validation curve.

NIRS sample preparation

Samples were prepared by grinding at 16,000 RPM with a Retsch Type ZM200 grinder (Haan, Germany) with a 1-millimeter screen. This ultra-centrifugal mill combines impact and shearing forces to reduce particle size, while adding very little to no heat to the sample, as it is quickly being ground and air cooled. After each sample was ground, the grinder components were thoroughly cleaned using a professional wet/dry vacuum (Rigid, model WD 14500, St Elyria, OH) and absorbent towelettes (Kimwipes, Kimberly-Clark, Roswell GA). Ground samples were labeled and placed into freezer-proof Ziploc bags (SC Johnson and Sons, Inc., Racine, WI) and stored at -20 degrees Celsius.

Prior to scanning, frozen samples or freshly ground samples were given adequate time to reach room temperature in the room housing the MPA: FT-NIR Spectrometer. Sample bags were thoroughly mixed to ensure a homogenous sample was deposited into the sample cup. The NIRS sample cup was filled so that no light could be seen from the bottom; samples were not packed into the cup.

Ingredient samples were prepared for NIRS analysis on “as is” basis. Although drying the samples would limit the moisture content of the samples and minimize spectral interference cause by water molecules, diet formulation and preparation are done on “as is” rather than a dry matter basis.

Experiments 3 through 7

These experiments followed the same procedures of Experiment 2, but the ingredient types for the calibration and validation curves became increasingly more specific. In Experiment 3, NIRS calibration and validation curves were made for commonly used animal by-products in poultry diets and consisted mainly of meat and bone meal (porcine and bovine) samples but also

included some poultry by-product meal and poultry tankage samples. In Experiment 4, NIRS calibration and validation curves were made for DDG(S) and consisted mainly of corn DDG and DDGS samples which had either low or high oil contents from the ethanol production process as well as a few DDGS samples from the alcoholic beverage production process. In Experiment 5, NIRS calibration and validation curves were made for bakery meal samples. Bakery meal is notoriously variable in composition (Waldroup et al., 1982; Dale et al., 1990). The samples utilized in the present research included a majority of general bakery meals, some ingredient specific cookie meals and cereal meals and a few all vegetable bakery meals. In Experiment 6, NIRS calibration and validation curves were made for SBM, and the samples included mostly solvent extracted SBM samples and some expeller (mechanically) extracted samples. Finally, in Experiment 7, NIRS calibration and validation curves were made for corn.

Statistical Analysis

Experiment 1. JMP® Pro 15 software (SAS Institute Inc. Cary NC) was used for regression analysis to show the correlation between the change in TME and nitrogen balance. The regression was considered significant at a P-value of 0.05 or less.

Experiments 2 through 7. All statistical analyses of the calibration and validation data sets were completed by using the Bruker OPUS Version 8.5 software package. Descriptions of the statistic measures used to evaluate NIRS calibration and validation curves were discussed previously in Chapter 2 of the dissertation.

CHAPTER 5

RESULTS

Experiment 1 –Nitrogen correction of TME

The N balance (nitrogen intake from the feed ingredient minus fecal nitrogen content) ranged from negative 1.95 to positive 1.14 grams (Figure 5.1). Roosters fed soy-based feed ingredients, which included full fat SBM, solvent and mechanically extracted SBM, and isolated soy protein products for the TME bioassay remained in a negative N balance (Figure 5.2), despite the high crude protein content of these ingredients. In contrast, when feeding animal by-product ingredient samples, which included feather meal, poultry tankage, poultry by-product meal, and bovine and porcine meat and bone meal samples, the roosters remained in a negative N balance for 45 percent of the samples, but the roosters had a positive N balance for 55 percent of the samples fed (Figure 5.3).

Because TME values are typically corrected for N balance to yield TME_N values, there can be a large difference between a feed ingredient's TME and TME_N values, as the N balance values become less than negative 1.5 or greater than zero (Figures 5.1 to 5.3 and Table 5.1). For all tested feed ingredients that had crude protein values above 40%, an isolated soy protein sample had the most negative N balance which was negative 0.60 and a feather meal sample had the highest positive N balance value of 1.14 (Table 5.2). As N balance increased, the difference between TME minus TME_N increased (Figure 5.3 and Table 5.2).

The endogenous control roosters for the TME_N bioassay are not fed a feed ingredient sample and thus, the initial 30 hour fast to clear the digestive tract is continued for another 42

Figure 5.1. The relationship ($R^2 = 0.9733$; $P < 0.0001$) between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and the difference between true metabolizable energy (TME) minus N-corrected TME (TME_N) for 762 ingredient samples on an as fed basis (Experiment 1).

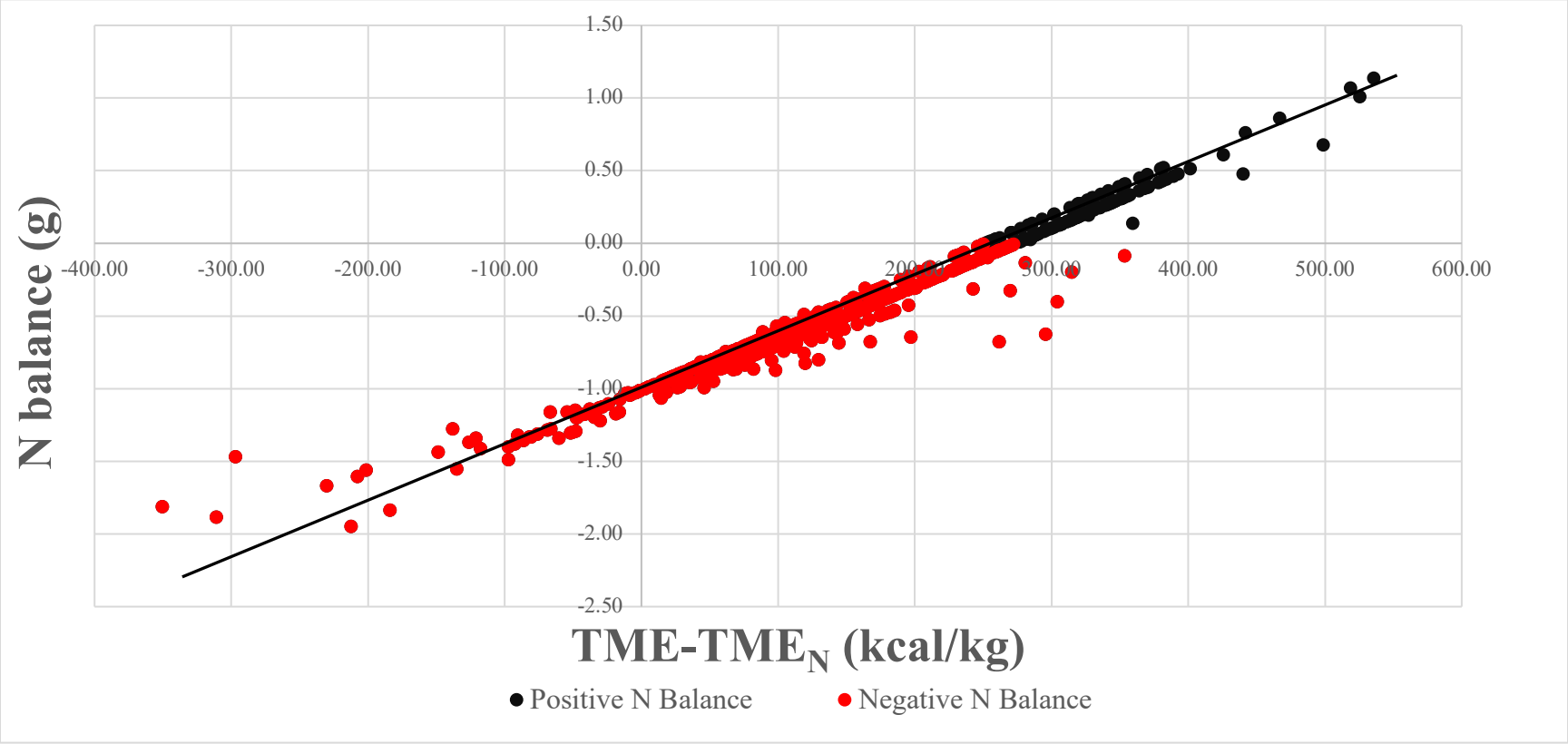


Figure 5.2. The relationship ($R^2 = 0.9808$; $P < 0.0001$) between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and the difference between true metabolizable energy (TME) minus N-corrected TME (TME_N) for 98 soy product ingredient samples including isolated soy samples on an as fed basis (Experiment 1).

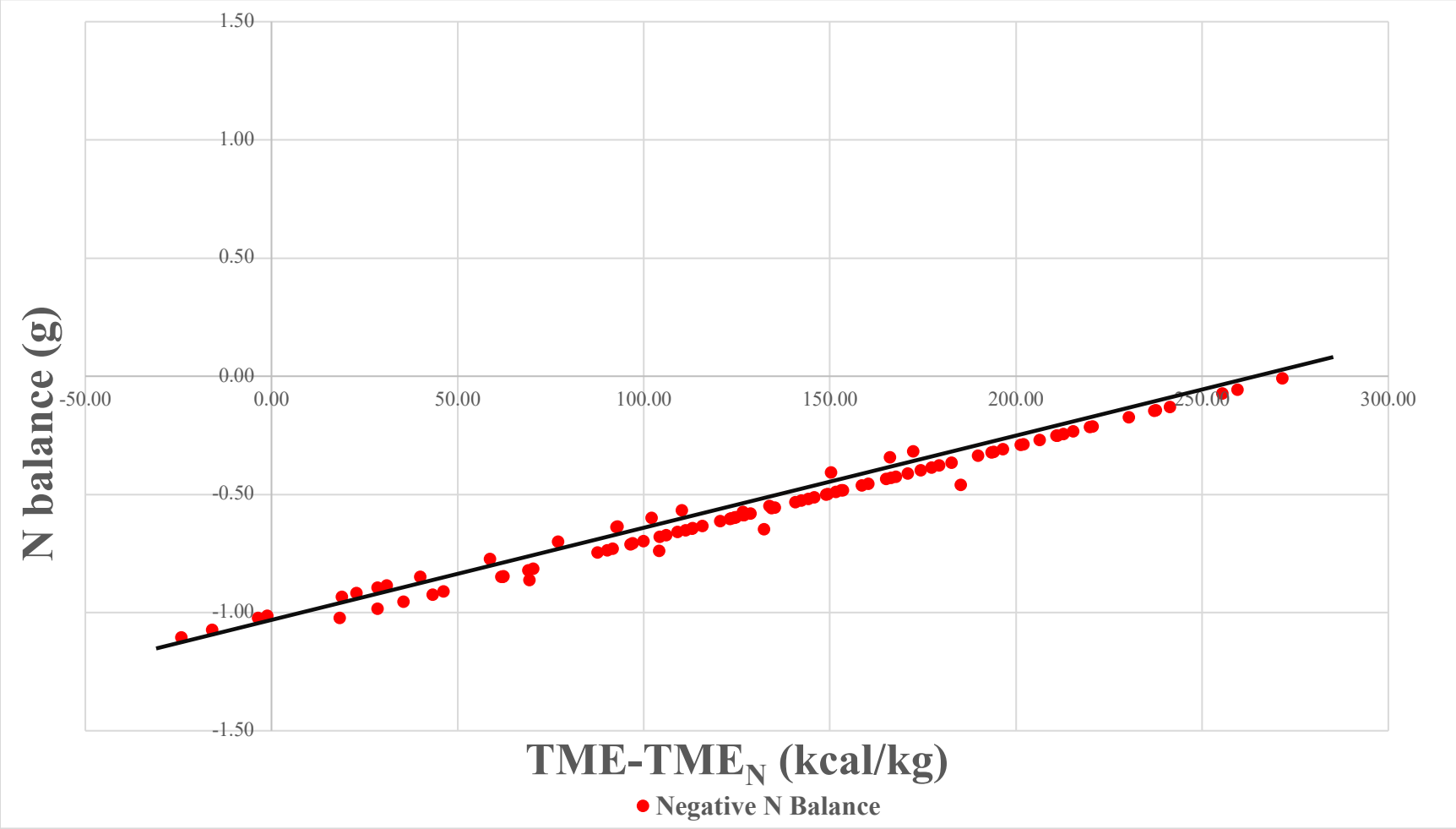


Figure 5.3. The relationship ($R^2 = 0.9703$; $P < 0.0001$) between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and the difference between true metabolizable energy (TME) minus N-corrected TME (TME_N) for 198 animal by-product feed ingredient samples on an as fed basis (Experiment 1).

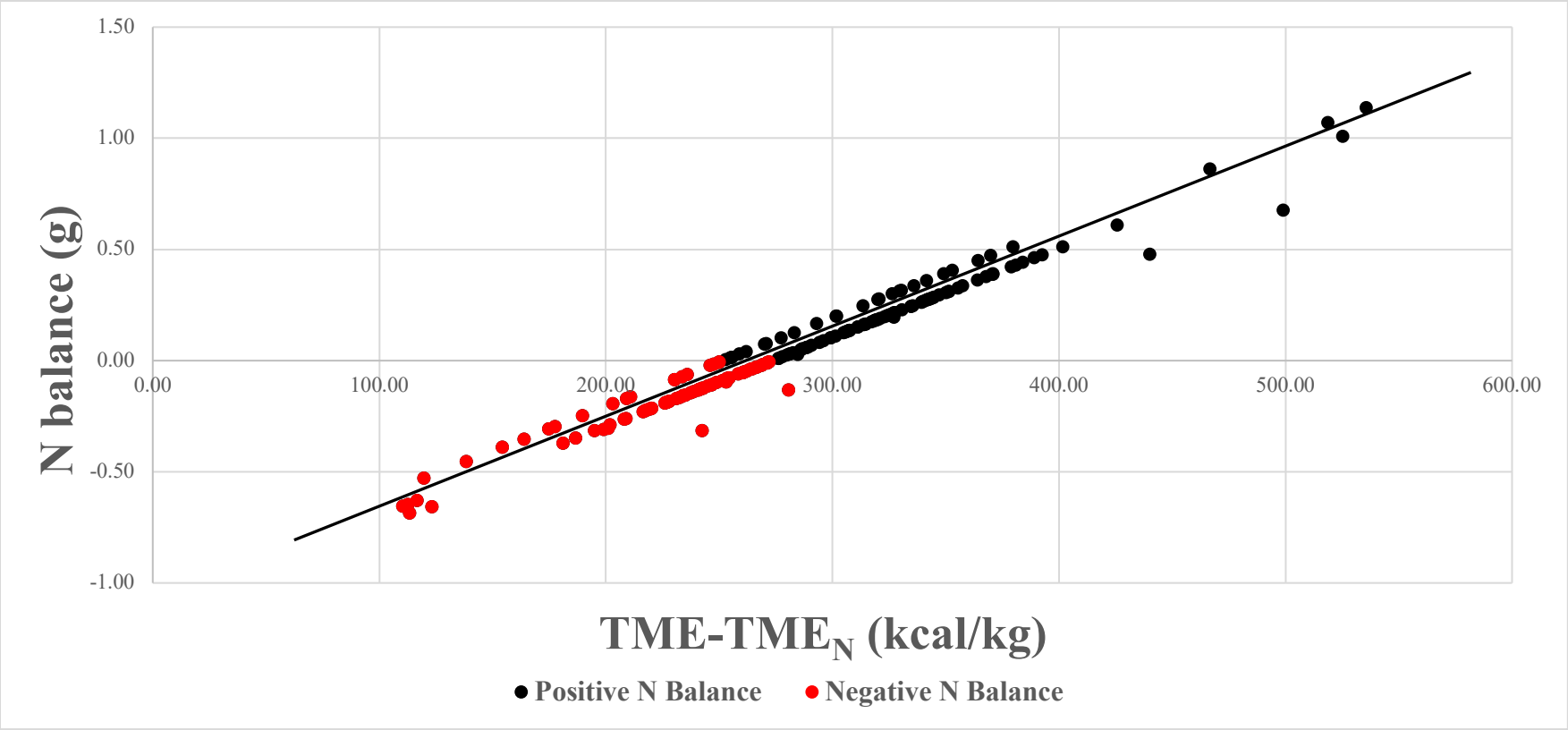


Table 5.1. The percent difference between determined true metabolizable energy (TME) values and nitrogen (N) corrected true metabolizable energy (TME_N) values (Experiment 1).

762 Total samples		
$TME-TME_N$ as % of TME	Number of samples	% of total samples
Greater than 10%	75	10
Less than 10%	687	90
Less than 5%	415	54
Less than 2.5%	150	20

Table 5.2. The relationship between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and true metabolizable energy (TME) and N-corrected true metabolizable energy (TME_N) values for select high protein samples (Experiment 1).

Sample	Crude Protein	N Balance	TME	TME_N	TME-TME_N	Difference[2]
	g	g	kcal/kg	kcal/kg	kcal/kg	%
Soy protein isolate	48.1	-0.60	2,936	2,811	125	4.25
Soy protein isolate	54.4	-0.24	2,973	2,760	213	7.15
Meat and bone meal	49.6	0.19	3,171	2,851	320	10.10
Meat and bone meal	55.9	0.31	2,952	2,602	350	11.86
Bovine plasma	77.4	0.68	3,340	2,841	499	14.94
Feather meal	81.9	1.14	4,135	3,599	536	12.95

[1]The difference between TME and TME_N as a percent of TME.

hours, which results in them having a N balance of negative 1.10 grams based on the N content of their feces collected during the 42-hour feces collection period. Interestingly, 50 of the 762 samples fed in the TME_N rooster bioassay resulted in the roosters having a negative N balance greater than the endogenous controls (data not shown). As the N balance continues to become more negative relative to the endogenous controls, the N corrected TME_N values actually become larger than the TME values (Table 5.3).

NIRS pre-processing

The pre-processing algorithms used by the OPUS® software package for optimizing the sample spectra calibration curves varied depending on the sample set (Table 5.4).

Experiment 2 – NIRS all poultry feed ingredients

The NIRS calibration curve for all poultry feed ingredients contained 150 samples and the calibration curve was validated with 551 samples (Table 5.5) for a total of 701 samples. This total number of samples is less than the 762 samples used in Experiment 1, and this is because some of the TME samples used in Experiment 1 were completed before the NIRS multi-purpose analyzer was purchased. In addition, some of the TME_N values were determined on whole grain samples for the cereal grains instead of ground samples, and the TME_N values for whole and ground cereal grains are not equal. These whole grain samples were not included for the NIRS determination where grinding of the samples is critical for accuracy. The all-encompassing TME_N NIRS calibration curve had an R^2 value of 0.75 (Table 5.6). The validation curve had an R^2 value of 0.95 and an RPD of 4.43 (Table 5.7). Of the samples included in the validation curve, 97% of the predicted TME_N values deviated less than plus or minus 10% from their determined TME_N values. For the 18 samples which had predicted values deviate more than 10%

Table 5.3. The relationship between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and true metabolizable energy (TME) and N-corrected true metabolizable energy (TME_N) values for select ingredients where the roosters fed the ingredient in the TME bioassay had a negative N balance that exceeded the endogenous control roosters negative N balance of 1.10g (Experiment 1).

Sample	Crude Protein	N Balance	TME	TME_N	TME-TME_N	Difference[2]
	g	g	kcal/kg	kcal/kg	kcal/kg	%
Soy hulls	11.9	-1.95	722	934	-212	29.36
Calliandra leaves	20.5	-1.67	678	909	-231	34.07
Sweet potato leaves	23.1	-1.60	771	979	-208	26.97
Soy hulls	12.6	-1.41	540	658	-118	21.85
Soy hulls	10.3	-1.29	709	777	-68	9.59
Soy hulls	11.9	-1.23	808	853	-45	5.56
Rice hulls	2	-1.19	527	561	-34	6.45
Soy hulls	10.18	-1.07	683	672	11	1.61

[1]The difference between TME and TME_N as a percent of TME.

Table 5.4. Near infrared reflectance spectroscopy pre-processing algorithms used for the sample spectra in each calibration curve developed for the prediction of nitrogen corrected true metabolizable energy (TME_N) of poultry feed ingredients.

Calibration Curve	Pre-processing algorithm
All-ingredients (Experiment 2)	Second derivative
Animal products[1] (Experiment 3)	Min-Max normalization
DDG(S)[2] (Experiment 4)	First derivative + Straight line subtraction
Bakery meal[3] (Experiment 5)	First derivative + Straight line subtraction
Soybean meal[4] (Experiment 6)	Straight line subtraction
Corn[5] (Experiment 7)	First derivative + Straight line subtraction

[1]Animal products commonly used as a poultry feed ingredient, and this included meat and bone meal (includes both porcine and bovine samples), poultry byproduct meal and poultry tankage.
[2]This calibration curve contained distiller's dried grains (DDG), distiller's dried grain with solubles (DDGS) from both ethanol and alcoholic beverage production, low and high oil DDG(S) and high protein DDG(S) samples.
[3]The bakery meal samples included general bakery meal, ingredient specific cookie and cereal meal, and all vegetable bakery meal samples.
[4]The majority of the soybean meal samples were solvent extracted, but mechanically processed and organic soybean meal samples were included as well as soybean meal made from genetically modified cultivars.
[5]The corn samples contained mostly standard commercial products, but also included organically grown corn samples and genetically modified cultivars.

Table 5.5. The nitrogen corrected true metabolizable energy (TME_N) content of feed ingredients used in the near infrared reflectance spectroscopy (NIRS) calibration and validation curves (Experiment 2).

Ingredient Type	Sample number		Mean TME_N		Minimum TME_N		Maximum TME_N	
	Calibration	Validation	Calibration	Validation	Calibration	Validation	Calibration	Validation
	n				kcal/kg			
Bakery meal	5	62	3704	3477	3465	2920	3844	4020
Canola	4	14	2424	2165	2259	1853	2779	2663
Corn	3	60	3398	3381	3251	3145	3623	3627
DDGS[1]	17	82	2609	2680	2017	2098	3175	3347
Feather meal	6	10	3599	3321	3403	2668	4008	3628
MBM[2]	15	108	2565	2717	2226	1945	2302	3318
PBM[3]	6	25	4189	3028	2248	2266	5588	4523
SBM[4]	2	69	2808	2701	2675	2328	2941	3267
Isolated soy	4	21	2554	2729	2027	2243	2960	3098
Fermented products	1	9	2642	2684	2642	2495	2642	2840
Peanut meal	2	5	3552	2843	3285	2562	3819	3320
Poultry tankage	10	16	3974	4458	2977	3850	4816	4717
Sorghum	1	9	3443	3269	3443	3006	3443	3611
Soy hulls	6	9	689	737	553	593	844	814
Wheat middlings	13	1	1954	2022	1814	2022	2065	2022
Other ingredients[5]	55	51	2342	2890	533	1069	4745	5142
Total	150	551						

[1]Includes distiller's dried grains (DDG), distiller's dried grain with solubles (DDGS) from both ethanol and alcoholic beverage production, low and high oil DDG(S) and high protein DDG(S) samples.
[2]Meat and bone meal (MBM) includes both porcine and bovine samples.
[3]Poultry byproduct meal (PBM).
[4]Included solvent extracted soybean meal (SBM) and expeller (mechanically) extracted soybean meal.
[5]Ingredients with 6 or less total samples from the calibration and validation curves combined, and they include: alfalfa meal, almond shells, arrow root leaves, black soldier fly larvae, blood meal, Brazil nut powder, calliandra leaves, carinata meal, cassava leaves, corn germ, corn gluten feed, cottonseed meal, dried grape pomace, experimental MBM based blend, fish meal, flaxseed meal, flaxseed protein product, hatchery waste meal, high protein rice product, jatropha meal, moringa powder, oats, peanut expeller meal, rice bran, rice hulls, experimental soy-based blend, spinach powder extract, sugarbeet root powder, sunflower meal, supermarket byproduct, sweet potato leaves, yeast cells.

Table 5.6. Descriptive statistics of the near infrared reflectance spectroscopy (NIRS) calibration curve for nitrogen corrected true metabolizable energy ($TME_{N.}$) for all poultry feed ingredients (Experiment 2).

Parameter	TME_N Calibration	
	NIRS	Determined[1]
Samples (n)	150	150
Mean (kcal/kg)	2656	2656
Minimum (kcal/kg)	533	588
Maximum (kcal/kg)	5588	4853
SD[2]	730	786
SEE[3]	531	
R^2	0.75	
RMSEE[4]	539	
RPD[5]	1.99	

[1]TME_N values determined by the method of Dale and Fuller (1984).
[2]Standard deviation.
[3]Standard error of the estimate.
[4]Root mean square error of the estimate.
[5]Residual predictive deviation.

Table 5.7. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for all poultry feed ingredients. (Experiment 2).

Predicted value/determined value (%)	Number of samples
< 90.00	9
90.00 - 92.49	27
92.50 – 94.99	58
95.00 – 97.49	99
97.50 – 99.99	101
100.00 – 102.49	99
102.50 – 104.99	61
105.00 – 110.00	88
> 110	9

Statistics

Parameter	NIRS	Determined[1]
Number of samples (n)	551	551
Mean (kcal/kg)	2932	2920
Minimum value (kcal/kg)	593	616
Maximum value (kcal/kg)	5142	4829
SD[2]	586	620
SEP[3]	142	
R^2	0.95	
RMSEP[4]	143	
RPD[5]	4.34	

Absolute percentage deviation

Percent of NIRS predicted values within ± 2.5% of determined value	36.1
Percent of NIRS predicted values within ± 5% of determined value	65.3
Percent of NIRS predicted values within ± 10% of determined value	96.7

[1]TME_N values determined by the method of Dale and Fuller (1984).
[2]Standard deviation.
[3]Standard error of the prediction.
[4]Root mean square error of the prediction.
[5]Residual predictive deviation.

from their determined value, 17 of them had a deviation less than 14% from their determined value, while the remaining sample deviated 20% from its determined value.

Experiment 3

The NIRS calibration and validation curves for commonly used animal by-products in poultry diets contained 97 and 79 samples respectively (Table 5.8). The calibration curve had an R^2 value of 0.81 (Table 5.9). The validation curve had an exceptional RPD value of 8.52 (Table 5.11), and 100% of the predicted values deviated less than 5% from their actual values (Table 5.11).

Experiment 4

The NIRS calibration and validation curves for DDG(S) contained 65 and 34 samples, respectively (Table 5.8). The calibration had an R^2 value of 0.81 (Table 5.9). Although the validation curve for DDG(S) had a lower RPD value than the animal by-product validation curve (Table 5.12), it still predicted 100% of the samples with less than 5% deviation from their determined values (Table 5.12).

Experiment 5

The NIRS calibration and validation curve for the determination of TME_N in bakery meal contained 40 and 27 samples, respectively (Table 5.8). The bakery meal calibration curve had an R^2 value of 0.88 (Table 5.9). Despite bakery meal being highly variable in composition, the calibration curve predicted 100% of the validation samples with a deviation of 2.5% or less from their determined values (Table 5.13).

Experiment 6

The NIRS calibration curve for soybean meal contained 43 samples while the validation curve contained 48 samples. The calibration curve had an R^2 value of 0.76 (Table 5.10). The

Table 5.8. Nitrogen corrected true metabolizable energy content of the ingredients used in the near infrared reflectance spectroscopy (NIRS) calibration and validation curves (Experiments 3 to 7).

Ingredient type	Sample number		Mean TME_N		Minimum TME_N		Maximum TME_N	
	Calibration	Validation	Calibration	Validation	Calibration	Validation	Calibration	Validation
	n				kcal/kg			
Animal products[1] (Experiment 3)	97	79	3002	3124	1945	2298	5588	4562
DDG(S)[2] (Experiment 4)	65	34	2702	2611	2098	2017	3347	3230
Bakery meal (Experiment 5)	40	27	3493	3496	2920	3161	4020	3949
Soybean meal (Experiment 6)	43	48	2625	2641	2328	2432	3267	3170
Corn (Experiment 7)	38	25	3375	3398	3227	3263	3627	3597

[1]Animal products commonly used as a poultry feed ingredient, and this included meat and bone meal (includes both porcine and bovine samples), poultry byproduct meal and poultry tankage.

[2]This calibration curve contained distiller's dried grains (DDG), distiller's dried grain with solubles (DDGS) from both ethanol and alcoholic beverage production, low and high oil DDG(S) and high protein DDG(S) samples.

[3]The bakery meal samples included general bakery meal, ingredient specific cookie and cereal meal, and all vegetable bakery meal samples.

[4]The majority of the soybean meal samples were solvent extracted, but mechanically processed and organic soybean meal samples were included as well as soybean meal made from genetically modified cultivars.

[5]The corn samples contained mostly standard commercial products, but also included organically grown corn samples and genetically modified cultivars.

Table 5.9. Descriptive statistics of the near infrared reflectance spectroscopy (NIRS) calibration curves for nitrogen corrected true metabolizable energy ($TME_{N.}$) for commonly used animal by-products (Experiment 3), dried distiller's grains with and without solubles [DDG(S)] (Experiment 4), and bakery meal (Experiment 5).

Parameter	Animal products[1]		DDG(S)[2]		Bakery meal[3]	
	NIRS	Determined[4]	NIRS	Determined[4]	NIRS	Determined[4]
Samples (n)	97	97	65	65	40	40
TME_N Mean (kcal/kg)	3002	3002	2702	2702	3493	3493
TME_N Minimum (kcal/kg)	2020	1945	2241	2098	2975	2920
TME_N Maximum (kcal/kg)	5371	5588	3434	3347	4046	4020
SD[5]	635	694	285	316	250	234
SEE[6]	282		137		89	
R^2	0.81		0.81		0.88	
RMSEE[7]	145		147		96.5	
RPD[8]	2.29		2.32		2.86	

[1]Animal products commonly used as a poultry feed ingredient, and this included meat and bone meal (includes both porcine and bovine samples), poultry byproduct meal and poultry tankage.
[2]This calibration curve contained distiller's dried grains (DDG), distiller's dried grain with solubles (DDGS) from both ethanol and alcoholic beverage production, low and high oil DDG(S) and high protein DDG(S) samples.
[3]The bakery meal samples included general bakery meal, ingredient specific cookie and cereal meal, and all vegetable bakery meal samples.
[4]TME_N values determined by the method of Dale and Fuller (1984).
[5]Standard deviation.
[6]Standard error of the estimate.
[7]Root mean square error of the estimate.
[8]Residual predictive deviation.

Table 5.10. Descriptive summary of the near infrared reflectance spectroscopy (NIRS) calibration curves for nitrogen corrected true metabolizable energy (TME_N) for soybean meal (Experiment 6) and corn (Experiment 7).

Parameter	Soybean meal[1]		Corn[2]	
	NIRS	Determined[3]	NIRS	Determined[3]
Samples (n)	43	43	38	38
TME_N Mean (kcal/kg)	2625	2625	3375	3375
TME_N Minimum (kcal/kg)	2479	2328	3268	3227
TME_N Maximum (kcal/kg)	3251	3267	3566	3627
SD[3]	207	237	74	101
SEE[4]		118		70
R^2		0.76		0.56
RMSEE[5]		118		68.8
RPD[6]		2.03		1.51

[1]The majority of the soybean meal samples were solvent extracted, but mechanically processed and organic soybean meal samples were included as well as soybean meal made from genetically modified cultivars.
[2]The corn samples contained mostly standard commercial products, but also included organically grown corn samples and genetically modified cultivars.
[3]TME_N values determined by the method of Dale and Fuller (1984).
[4]Standard deviation.
[5]Standard error of the estimate.
[6]Root mean square error of the estimate.
[7]Residual predictive deviation.

Table 5.11. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for animal by-products (Experiment 3).

Predicted value/determined value (%)	Number of samples
< 90.00	0
90.00 - 92.49	0
92.50 – 94.99	0
95.00 – 97.49	8
97.50 – 99.99	31
100.00 – 102.49	26
102.50 – 104.99	13
105.00 – 107.49	0
107.50 – 110.00	0
> 110	0

Statistics

Parameter	NIRS	Determined[1]
Number of samples (n)	79	79
Mean (kcal/kg)	3125	3124
Minimum value (kcal/kg)	2312	2298
Maximum value (kcal/kg)	4469	4562
SD[2]	575	597
SEP[3]	68	
R^2	0.99	
RMSEP[4]	69.6	
RPD[5]	8.52	

Absolute percentage deviation

Percent of NIRS predicted values within ± 2.5% of determined value	73.1
Percent of NIRS predicted values within ± 5% of determined value	100.0
Percent of NIRS predicted values within ± 10% of determined value	100.0

[1]TME_N values determined by the method of Dale and Fuller (1984).
[2]Standard deviation.
[3]Standard error of the prediction.
[4]Root mean square error of the prediction.
[5]Residual predictive deviation.

Table 5.12. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for distiller's dried grains with and without solubles (Experiment 4).

Predicted value/determined value (%)	Number of samples
< 90.00	0
90.00 - 92.49	0
92.50 – 94.99	0
95.00 – 97.49	0
97.50 – 99.99	4
100.00 – 102.49	16
102.50 – 104.99	9
105.00 – 107.49	5
107.50 – 110.00	0
> 110	0

Statistics		
Parameter	NIRS	Determined[1]
Number of samples (n)	34	34
Mean (kcal/kg)	2602	2611
Minimum value (kcal/kg)	2072	2017
Maximum value (kcal/kg)	3175	3230
SD[2]	263	266
SEP[3]	57	
R^2	0.95	
RMSEP[4]	55.8	
RPD[5]	4.75	

Absolute percentage deviation	
Percent of NIRS predicted values within ± 2.5% of determined value	73.5
Percent of NIRS predicted values within ± 5% of determined value	100.0
Percent of NIRS predicted values within ± 10% of determined value	100.0

[1]TME_N values determined by the method of Dale and Fuller (1984).
[2]Standard deviation.
[3]Standard error of the prediction.
[4]Root mean square error of the prediction.
[5]Residual predictive deviation.

corresponding validation curve had an exceptional RPD value of 8.21, and all of the validation samples had predicted values that deviated less than 2.5% from their determined values (Table 5.14).

Experiment 7

The corn calibration and validation curves had the least number of samples relative to the previous curves with only 38 samples in the calibration and 25 samples in the validation (Table 5.8). The calibration curve had an R^2 value of 0.56 (Table 5.10). Despite the lower R^2 value relative to the other calibration curves, the corn NIRS calibration curve for TME_N predicted all of the validation samples with 2.5% or less deviation from their actual values (Table 5.15)

Table 5.13. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for bakery meal (Experiment 5).

Predicted value/determined value (%)	Number of samples
< 90.00	0
90.00 - 92.49	0
92.50 – 94.99	0
95.00 – 97.49	0
97.50 – 99.99	14
100.00 – 102.49	13
102.50 – 104.99	0
105.00 – 107.49	0
107.50 – 110.00	0
> 110	0

Statistics

Parameter	NIRS	Determined[1]
Number of samples (n)	27	27
Mean (kcal/kg)	3495	3496
Minimum value (kcal/kg)	3145	3161
Maximum value (kcal/kg)	3876	3949
SD[2]	175	178
SEP[3]	44	
R^2	0.93	
RMSEP[4]	40.6	
RPD[5]	3.78	

Absolute percentage deviation

Percent of NIRS predicted values within ± 2.5% of determined value	100.0
Percent of NIRS predicted values within ± 5% of determined value	100.0
Percent of NIRS predicted values within ± 10% of determined value	100.0

[a]TME_N values determined by the method of Dale and Fuller (1984).
[b]Standard deviation.
[c]Standard error of the prediction.
[d]Root mean square error of the prediction.
[e]Residual predictive deviation.

Table 5.14. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for soybean meal (Experiment 6).

Predicted value/determined value (%)	Number of samples
< 90.00	0
90.00 - 92.49	0
92.50 – 94.99	0
95.00 – 97.49	0
97.50 – 99.99	16
100.00 – 102.49	32
102.50 – 104.99	0
105.00 – 107.49	0
107.50 – 110.00	0
> 110	0

Statistics

Parameter	NIRS	Determined[1]
Number of samples (n)	48	48
Mean (kcal/kg)	2648	2641
Minimum value (kcal/kg)	2445	2432
Maximum value (kcal/kg)	3169	3170
SD[2]	221	217
SEP[3]	26	
R^2	0.98	
RMSEP[4]	27.1	
RPD[5]	8.21	

Absolute percentage deviation

Percent of NIRS predicted values within ± 2.5% of determined value	100.0
Percent of NIRS predicted values within ± 5% of determined value	100.0
Percent of NIRS predicted values within ± 10% of determined value	100.0

[a]TME_N values determined by the method of Dale and Fuller (1984).
[b]Standard deviation.
[c]Standard error of the prediction.
[d]Root mean square error of the prediction.
[e]Residual predictive deviation.

Table 5.15. Mathematical evaluation of the near infrared reflectance spectroscopy (NIRS) validation curve for nitrogen corrected true metabolizable energy (TME_N) when using the corresponding calibration prediction curve for corn (Experiment 7).

Predicted value/determined value (%)	Number of samples
< 90.00	0
90.00 - 92.49	0
92.50 – 94.99	0
95.00 – 97.49	0
97.50 – 99.99	16
100.00 – 102.49	9
102.50 – 104.99	0
105.00 – 107.49	0
107.50 – 110.00	0
> 110	0

Statistics

Parameter	NIRS	Determined[1]
Number of samples (n)	25	25
Mean (kcal/kg)	3396	3398
Minimum value (kcal/kg)	3282	3263
Maximum value (kcal/kg)	3563	3597
SD[2]	82	94
SEP[3]	25	
R^2	0.91	
RMSEP[4]	26.9	
RPD[5]	3.43	

Absolute percentage deviation

Percent of NIRS predicted values within ± 2.5% of determined value	100.0
Percent of NIRS predicted values within ± 5% of determined value	100.0
Percent of NIRS predicted values within ± 10% of determined value	100.0

[a]TME_N values determined by the method of Dale and Fuller (1984).
[b]Standard deviation.
[c]Standard error of the prediction.
[d]Root mean square error of the prediction.
[e]Residual predictive deviation.

CHAPTER 6

DISCUSSION

Nitrogen correction of TME

The current research emphasizes that the original nitrogen correction for most poultry feed ingredients is still appropriate. Even though about half of the animal products tested were observed to be in a positive nitrogen balance, the difference in TME on a percentage basis did not exceed 15%, which is in line with what Dale and Fuller observed (1984). The negative nitrogen balance observed in all isolated soy and soybean meal products during the assay period is most likely due to plant protein sources being less anabolic when compared to animal protein sources which proportionally, have superior essential amino acid content (Duodu et al., 2003; Berrazaga et al., 2019). Although the essential amino acid profile of plant-based protein sources is compositionally variant from that of animal products and less complementary to the dietary amino acid requirements of broilers, the majority of dietary protein in poultry feed formulas is derived from plant proteins, predominantly in the form of soybean meal (Beski et al. 2015). Another factor that contributes to the low bioavailability of plant proteins is the presence of antinutritional factors such as phytic acid and non-starch polysaccharides that bind proteins and impede the access of key enzymes, thus making them unavailable for digestion (Cheryan & Rackis, 1980; Berrazaga et al., 2019). Even though animal protein sources are well balanced in terms of essential amino acids, they are typically used to complement the overall amino acid profile in the diet formula due to their expense (Akhter et al., 2008).

The majority of the samples that had a nitrogen balance that exceeded that of the endogenous control were high fiber ingredients such as soy hulls, rice hulls, and leafy materials. These ingredients were not fed in the original research but are now commonly fed in broiler breeder feed restriction programs for added bulk (Arrazola et al., 2020; Nascimento et al., 2021). Because these samples are low in energy (<1,500 kcal), adding to the metabolizable energy value is seen as an overestimation of the available energy in these feedstuffs. It was concluded from the current research that this addition to the TME_N for these samples is due to the samples' high fiber content in combination with the nitrogenous waste of cecal microbes. High fiber ingredients are typically used as dietary diluents due to their capacity to reduce nutrient availability (Mateos et al., 2012; J. Tejeda & K. Kim, 2021). As the fibrous material slowly makes its way through the digestive tract, it causes an increase in the sloughing of cells, which translates to excreta production that exceeds the amount of the sample fed on a per weight basis. Additionally, when undigested high fiber material enters the caeca, it is broken down and excreted along with some cecal microbes and their associated protein in the feces (Leung et al., 2018). These additions increase the overall nitrogen content of the excreta. An example of a feed ingredient that participates in this phenomenon is rice hulls, which have around 20% silica in their epidermal wall. This tough outer coating contributes significantly to their abrasive character, which increases the sloughing of cells as the hulls traverse through the gut (Park et al., 2003). These conditions contribute to a large negative nitrogen balance and impart the need for a significant nitrogen correction in the TME_N calculation for these feed ingredients.

In contrast to samples where the nitrogen correction deducts from the TME, these samples with a high non-digestible fiber content possess traits which mathematically translate to

the correction contributing to the TME value rather than diminishing it. Therefore, for samples where the nitrogen balance exceeds that of the endogenous controls, an adjustment has been proposed to the correction where it is applied up to the nitrogen balance of the endogenous controls of negative 1.10 grams of nitrogen and not past it. By doing this, calculated values are observed to be more realistic to the actual energy associated with these already low energy feedstuffs, leading to greater accuracy when determining metabolizable energy values (Table 6.1). For example, in a sample of soy hulls observed to have a difference in TME of 29% using the standard nitrogen correction calculation when determining TME_N, the difference was reduced to 1% when the revised correction was applied (Table 6.1). Figure 6.1 also indicates there is a better fit of nitrogen balance versus TME-TME_N following the adjustment of the nitrogen correction in these samples. Despite the overarching increase in accuracy provided by the revised correction, a portion of nitrogen for these samples is ultimately digestible, thus requiring further investigation to justify the inclusion of the digestible nitrogen content from the samples along with the revised adjustment.

NIRS all poultry feed ingredients

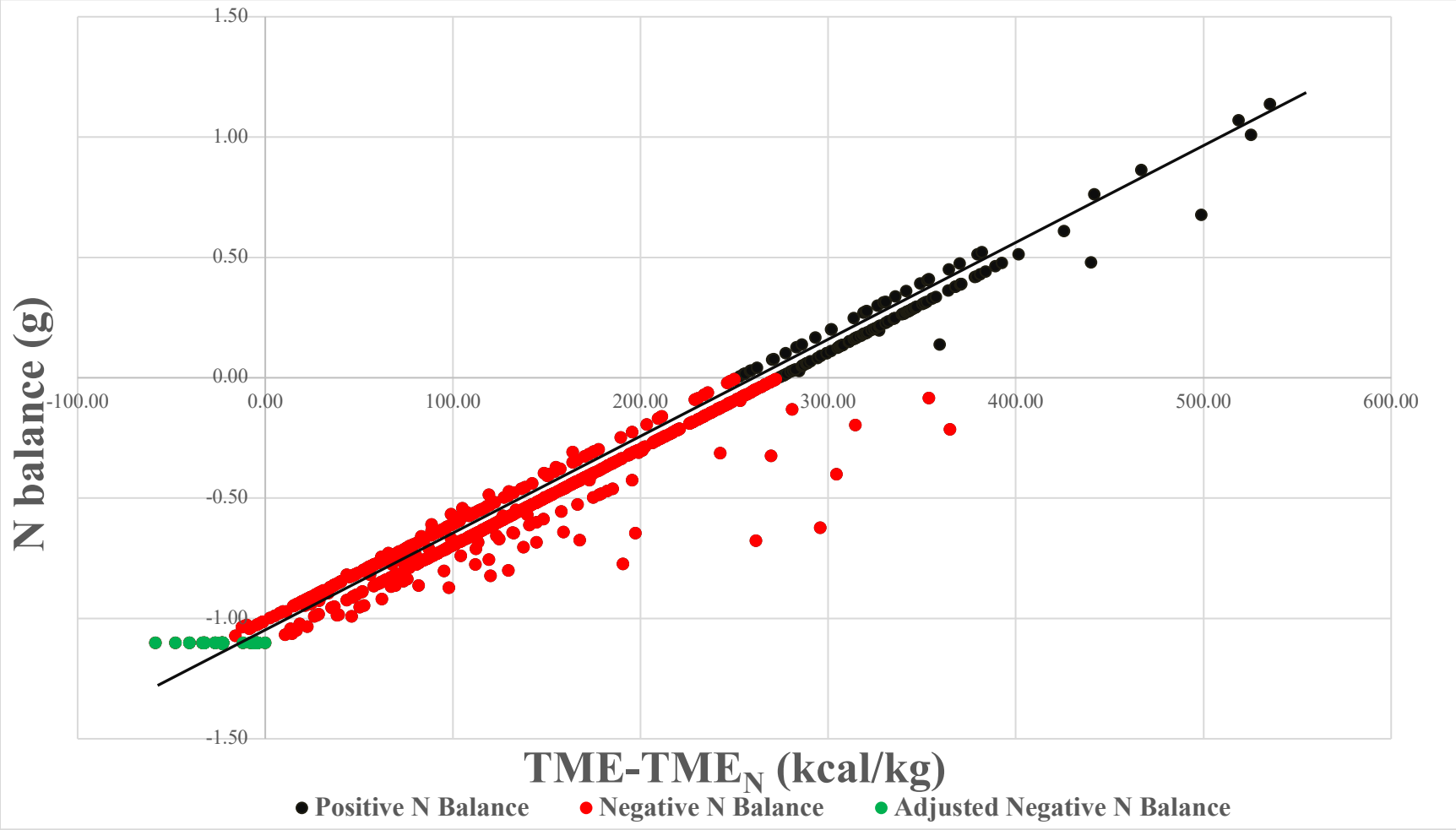
The current research resulted in the creation of an NIRS calibration curve designed to predict the TME_N of all poultry feed ingredients. This TME_N curve is of particular use to feed manufacturers who have newly developed or alternative feed ingredients that do not have substantial samples or data pools for the creation of an individual curve for that feedstuff. These manufacturers require reliable estimates to determine if the product is worth further exploration or how similar it may be to certain feed ingredients, thus this curve was designed with the intention of analyzing any potential feed ingredient for an initial assessment of its TME_N value.

Table 6.1. The relationship between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and true metabolizable energy (TME) and N-corrected true metabolizable energy (TME_N) values for select ingredients where the roosters fed the ingredient in the TME bioassay had a negative N balance that exceeded the endogenous control roosters negative N balance of 1.10g with the nitrogen correction adjustment made (Experiment 1).

Sample	Crude Protein	N Balance	TME	TME_N	TME-TME_N	Difference[1]	Adjusted TME_N	TME-TME_N	Difference[1]
	g	g	kcal/kg	kcal/kg	kcal/kg	%	kcal/kg	kcal/kg	%
Soy hulls	11.9	-1.95	722	934	-212	29.40	716	6	0.83
Calliandra leaves	20.5	-1.67	678	909	-231	33.98	696	-18	2.65
Sweet potato leaves	23.1	-1.60	771	979	-208	26.91	790	-19	2.46
Soy hulls	12.6	-1.41	540	658	-118	21.75	556	-16	2.96
Soy hulls	10.3	-1.29	709	777	-68	9.64	701	8	1.13
Soy hulls	11.9	-1.23	808	853	-45	5.62	800	8	0.99
Rice hulls	2	-1.19	527	561	-34	6.52	519	8	1.52
Soy hulls	10.18	-1.07	683	672	11	1.61			

[1]The difference between TME and TME_N as a percent of TME.

Figure 6.1. The relationship ($R^2 = 0.9799$; $P < 0.0001$) between nitrogen (N) balance (total N intake from ingredient fed minus total excreta N) and the difference between true metabolizable energy (TME) minus N-corrected TME (TME_N) for 762 ingredient samples on an as fed basis.



Although, the coefficient of determination appeared to be low for the NIRS calibration curve for all poultry feed ingredients, increased variability within the calibration set allowed the curve to the predict the TME_N of any given ingredient with accuracy and resulted in the R^2 being lower, thus increasing the overall adaptability of the curve. Through incorporating samples that encapsulate a plethora of variables, the validation curve was able to predict with accuracy, as denoted by the R^2 value of 0.95 and an RPD of 4.43. In previous research by Cope (2021), in which a calibration curve of 213 samples was created, the corresponding NIRS validation curve of 151 samples predicted only 79% of the samples with a deviation less than 10% of their determined value. In the current research with a NIRS calibration of only 150 samples, the associated NIRS validation curve of 551 samples predicted 97% of the TME_N values with a deviation of less than 10% of their determined TME_N values, with only 18 samples (3.3%) being outside the margin of plus or minus 10%.

Also in previously conducted research, samples not within plus or minus 10% were predominantly those with low TME_N values and high fiber content (Cope, 2021). However, this trend was a fundamental result of the nitrogen correction within the TME_N calculation. As presented in Experiment 1, the samples assayed, when fed to the roosters put them in a substantially more negative nitrogen balance when compared to the endogenous control roosters. For these samples, the TME_N calculation was adjusted to better estimate the metabolizable energy associated with the low energy feedstuffs. After the nitrogen correction adjustment, only 9 (50%) of the samples that were not within plus or minus 10% had TME_N values less than 1,500 kcal/kg.

Ingredient specific calibrations

The NIRS specific ingredient curves created in the current research were incredibly predictive with every single curve predicting the TME_N of each feedstuff sample within 5% of the laboratory determined value for each ingredient sample. The current research agrees with Cope (2021), which suggested that specific-ingredient calibration curves increase the predictability of TME_N when compared to an all-feed ingredient NIRS calibration curve. In contrast to previous research by Cope (2021), the current research includes at least 20 more samples in each individual ingredient sample set. Increasing the number of samples within an ingredient set can increase the robustness of an NIRS calibration curve, ultimately allowing the curve's predictability to improve (Lucà et al., 2017).

In contrast to the individual animal by-products and DDG(S) calibration curves which resulted in 100% of their validation samples deviating less than 5% from their actual bioassay determined values, when the all-ingredient calibration was used only 57% of the animal by-product validation samples and 50% of the DDG(S) validation samples deviated less than 5% from their actual bioassay determined values. Similarly, while the individual bakery meal, soybean meal and corn calibration curves resulted in 100% of their validation samples deviating less than 2.5% from their actual bioassay determined values, when the all-ingredient calibration was utilized for these ingredient categories only 52, 45 and 55 percent of the bakery meal, soybean meal and corn validation samples respectively, deviated less than 2.5% from their corresponding bioassay determined values. These results underscore the increased predictability of ingredient specific NIRS calibrations.

The low error of less than 5% from the ingredient specific NIRS calibration and validation curves created in this research shows that this technology to predict the TME_N of

specific poultry feed ingredients can be used for poultry diet formulation. For example, based on the NIRS TME_N calibration curve for bakery meal, the curve was more accurate in predicting the TME_N values than the commonly used prediction equations developed using proximate analysis values (Dale et al., 1990). When evaluating the samples as outlined by Dale et al. (1990), only 22% were able to be predicted within 2.5% of their determined value when using the equation, whilst the NIRS calibration curve in Experiment 5 predicted 100% of samples within 2.5% of their determined value. With this low error, formulating poultry diets using NIRS technology could reduce the need for over formulation as NIRS can better reflect the variation of nutrient content than an average book value intended to represent all sources of variability. The use of NIRS to evaluate feed ingredients has previously demonstrated production savings when diets were formulated using NIRS determined nutrient specifications rather than standard book values (Steed et al., 2020).

The current research on the prediction of TME_N values by NIRS technology agrees with Cope (2021) in that the traditional statistical evaluation such as R^2 and RPD of NIRS validation curves, provides an overall evaluation of a curve rather than on an individual sample basis. Though traditional statistics are important, for practical diet formulation, nutritionists must know how large the deviations are from the actual determined values. For example, even though the animal products NIRS validation curve had an R^2 of 0.99 and an RPD of 8.52, only 73% of samples were predicted within 2.5% of their determined value but 100% were predicted within 5% of their determined value. Nutritionists need to know such deviations to accurately compensate for any potential underestimation or overestimation of the metabolizable energy content of the feed ingredient determined by NIRS for accurate diet formulation.

Conclusions

The current research indicates that the nitrogen correction established by the original research on the TME_N rooster bioassay is appropriate for most of the poultry feed ingredients used today. However, for ingredients where the nitrogen balance exceeds that of the endogenous control, an adjustment is necessary to obtain accurate values for these poultry feed ingredients. The subsequent NIRS calibration and validation curve created for all poultry feed ingredients can be used for feed manufacturers with new alternative ingredients looking for a general estimate of the metabolizable energy for the feedstuff. In addition, this research agrees with previous research that individual ingredient NIRS calibration curves determine more precise TME_N values when compared to regression equations which can then be used for poultry diet formulation.

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