AMINO ACID DIGESTIBILITY OF VARIOUS FEEDSTUFFS USING DIFFERENT METHODS

BY

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DISSERTATION

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ABSTRACT

The protein requirement for poultry is actually a requirement for amino acids. Recently, there has been much interest in formulating diets on a digestible amino acid basis. The most accepted methods of determining amino acid digestibility are the precision-fed cecectomized rooster assay (PFR) and standardized ileal chick assay (SID). The PFR involves tube-feeding cecectomized adult roosters and excreta are then quantitatively collected for amino acids. For the SID, 3-week-old broiler chicks are ad libitum fed a semi-purified diet with the test ingredient added as a sole source of protein for a period of several days. The animals are then euthanized and ileal digesta are collected and analyzed for amino acids. When these two methods were compared for 15 feed ingredients, standardized amino acid digestibility was found to vary among feed ingredients and among samples of the same ingredient. There were generally no differences in amino acid digestibility for six corn and four distiller's dried grains with solubles (DDGS) samples between the two methods. The PFR did yield significantly ($P<0.05$) greater digestibilities for two other DDGS samples, a meat and bone meal sample, and a poultry by-product meal, whereas the SID yielded higher digestibility values for another meat and bone meal. A new method of determining amino acid digestibility was developed utilizing the precision-feeding of 3-week-old broiler chicks and collecting ileal digesta to measure amino acid digestibility. The new precision-fed ileal chick assay (PFC) involved fasting chicks for a period of at least eight hours, then precision-feeding 10 g of feed and subsequent collection of ileal digesta at four hours post-feeding. Amino acid digestibilities were standardized by precision-feeding a nitrogen-free diet and analyzing ileal digesta for amino acids. In order to determine the

validity of the new PFC, several feedstuffs were obtained and standardized amino acid digestibility was determined using the PFR, PFC and SID methods. Differences in amino acid digestibility were not consistent among methods and ingredients. For corn, the PFC yielded significantly greater values than the PFR and SID. For corn gluten meal, the PFR yielded higher values than the PFC and SID for majority of the amino acids. The PFR yielded higher digestibilities than the PFC for three DDGS samples evaluated. Digestibility values for soybean meal and meat and bone meal were found to be in general agreement for the three methods. There were some differences among methods for fish meal; however, these differences were not consistent among methods or amino acids. The results of these studies validate that the PFR, SID, and the new PFC are all acceptable for determining amino acid digestibility in poultry.

To Grace and Vicky

Thank you for all your love and support throughout the years

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TABLE OF CONTENTS

Chapter 1

LITERATURE REVIEW

INTRODUCTION

Amino acids are important components of poultry diets. They are in constant turnover in the body and without proper dietary intake; deficiencies can cause detrimental effects on growth and production. These amino acids play an important role in structural and protective tissues in the body and are also important in enzyme and tissue functions (NRC, 1994). Recently, there has been much interest in formulating diets on a digestible amino acid basis. Formulating diets in this fashion can result in a decrease of excess nutrients being excreted into the environment. Excess nitrogen excretion can cause detrimental environmental effects. Feed safety margins are commonly used in commercial feed formulations and reducing these safety margins can help reduce nutrient excretion into the environment. Reducing these feed safety margins can also decrease feed costs, which is an integral input in poultry production. However, there is a lack of information regarding the amino acid content and digestibility of commonly used feedstuffs (Applegate et al., 2008; Garcia et al., 2007) and there is a lack of consistency in the methods used to determine amino acid digestibilities.

AMINO ACIDS

Dietary requirements for protein are actually requirements for amino acids. Amino acids are the building blocks of protein and are the products of protein hydrolysis. There are over 20 amino acids in body protein and all are considered to be physiologically essential. However, poultry are unable to synthesize 10 of these amino acids, and therefore, must be supplied in the diet and are considered to be indispensable

amino acids (Ravindran and Bryden, 1999). These indispensable amino acids are lysine, methionine, tryptophan, thereonine, arginine, isoleucine, leucine, histidine, phenylalanine, and valine. Glycine is sometimes identified as being indispensable as well in modern broiler chickens since the rate of synthesis often cannot support the maximum growth rate of these birds. Tyrosine and cysteine are considered as 'semi-essential' since they can be formed in tissues from phenylalanine and methionine, respectively (Ravindran and Bryden, 1999). Hydroxylysine and hydroxyproline are other amino acids that have been detected in both poultry diets and intestinal digesta as well as lanthionine, orinthine, and taurine (Adedokun, 2007). In practical diets, methionine (or methionine + cysteine) has been shown to be the first limiting amino acid and lysine is the second limiting amino acid for growth and egg production for poultry (Ravindran and Bryden, 1999).

AMINO ACID ANALYSIS

Amino acid analysis is routinely carried out using ion-exchange chromatography. The chemical procedure for amino acid analysis involves four distinctly different steps. First, the protein is hydrolyzed, followed by separation, identification, and finally quantification of amino acids. The chemical procedure often used for amino acid analyses involves sample hydrolysis in a 6 N HCl for 24 h at 110 ºC under N atmosphere. For sulfur containing amino acids (methionine and cysteine), performic acid oxidation is first carried out before acid hydrolysis. This step is to convert cysteine to cysteic acid and methionine to methionine sulfone, which are acid stable and can then be separated by chromatographic methods. Samples for tryptophan analysis are hydrolyzed using barium or sodium hydroxide to avoid the destruction of tryptophan by acid hydrolysis and to enhance its stability. The amino acids in the hydrolysate are then usually determined by

HPLC after post-column derivatization (AOAC, 2000; 982.30 E [a,b,c]). Ambler (1981) reported that all four steps involved in analysis can introduce error and variability in results.

METHODOLOGY

Several terms are used in assessment of nutrient quality of feedstuffs, especially for proteins and amino acids. Bioavailable nutrients are important for efficient utilization for growth, maintenance, and production in animals. Nutrients, like amino acids, are considered to be bioavailable when they can be used for normal metabolic functions in the body. However, quantitative assessment of bioavailable protein and amino acids is difficult in comparison to carbohydrates and fats, which are primarily energy sources (Ravindran and Bryden, 1999). The main function of dietary protein is to provide amino acids and protein quality is measured by both the nitrogen content and amino acid constituents of the protein (NRC, 1994; Ravindran and Bryden, 1999). Therefore, bioavailability of amino acids is generally defined as the proportion of ingested dietary amino acids that are absorbed in a chemical form that renders these amino acids potentially suitable for metabolism or protein synthesis (Batterham, 1992; Lewis and Bayley, 1995). However, bioavailability cannot be measured easily. Slope-ratio methods have traditionally been used to measure whole body protein deposition or amino acid oxidation and to estimate amino acid bioavailability relative to a reference standard (Batterham, 1992; Moehn et al., 2005). Values determined through a slope-ration technique may underestimate bioavailability and represent relative values (Stein et al., 2007). Thus, amino acid digestibility has been suggested to be a better measure of bioavailability in feedstuffs because it is much easier to conduct digestibility assays than

slope-ratio assays (Batterham, 1992; Ravindran and Bryden, 1999; Parsons, 2002; Stein et al., 2007).

Digestibility and availability are often used interchangeably, but refer to different things (Ravindran and Bryden, 1999; Moughan, 2003). The term digestibility refers to the process of digestion and absorption and reflects enzymatic hydrolysis and microbial fermentation of ingested protein, peptides, and absorption of amino acids from the gastrointestinal lumen (Fuller, 2003).

APPARENT AMINO ACID DIGESTIBILITY

Amino acid digestibility values can be expressed several different ways; apparent digestibility, standardized digestibility, and true digestibility. Apparent digestibility values, from excreta or ileal digesta, are digestibility values that include and do not distinguish between both dietary and endogenous amino acids (Ravindran and Bryden, 1999). These values are not corrected for endogenous amino acid flow and can be influenced by the level of feed intake and dietary protein concentrations (Fan et al., 1994). Low protein and amino acid intakes may cause greater proportions of endogenous amino acids to be present in the digesta in relation to protein from dietary origin. When evaluating low protein assay diets, apparent amino acid digestibility will be underestimated due to high endogenous amino acid contribution. However, as protein intake increases, the proportion of endogenous amino acid sources will decrease and apparent digestibility has been found to approach that of true digestibility (McNab, 1989; Ravindran and Bryden, 1999; Stein et al., 2005; Stein et al., 2007). It should be noted that in swine nutrition, ileal amino acid digestibility is favored because it removes hindgut fermentation by microbes and is commonly used to evaluate feedstuffs (Stein et al., 2007).

ENDOGENOUS AMINO ACIDS

Along with dietary sources, proteins are also supplied to the gut in the form of endogenous secretions. Simon et al. (1983) reported that up to 25% of the daily protein synthesis may be secreted into the gut in various forms. Endogenous amino acids in the gastrointestinal tract originate from various sources. These endogenous proteins predominantly originate from various digestive secretions, such as saliva, bile, pancreatic secretions, gastric secretions, and intestinal secretions. Mucoproteins and desquamated intestinal epithelial cells also contribute to endogenous amino acids in the gut (Scott et al., 1982). Metabolites, in the form of peptides and free amino acids, released by protein catabolism from the lower gut can also contribute to the endogenous loss proteins (Simon et al., 1986). Significant losses of endogenous amino acids occur during the process of digestion and absorption along the gastrointestinal tract (Angkanaporn et al., 1996). It is important to estimate these losses since they are considered to be inevitable and necessary to standardize digestibility coefficients.

Total endogenous amino acid flow in the digestive tract is the sum of basal endogenous proteins and diet induced secretions. Basal endogenous losses represent the minimum quantities of amino acids inevitably lost by the animal which are related to the physical flow of feed in the gut, the animal's metabolic state, and thus are not affected by diet or feedstuff composition (Stein et al., 2007). Basal endogenous losses have also been referred to as nonspecific or diet-independent losses. Several researchers have reported that basal endogenous amino acid flow can be best established at feed intakes that are close to voluntary feed intake of animals and expressed in proportion to dry matter intake (DMI; Boisen and Moughan, 1996; Jansman et al., 2002). Diet specific

endogenous amino acids also contribute to the amino acid loss in the gut. The latter have also been referred to as extra or diet-dependent losses (Stein et al., 2007). These specific losses are in addition to basal losses that are induced by specific feed ingredient characteristics, such as fiber content and antinutritional factors (Schulze et al., 1995). For example, while high fiber and the presence of antinutritional factors can result in specific amino acid losses that are greater than 50% of the total endogenous amino acid flow that occurs from low-fiber diets containing no anti-nutritional factors (Moughan, 2003; Souffrant, 1991). Highly digestible purified protein sources minimize diet-specific or diet-dependent endogenous amino acid losses.

METHODS TO ESTIMATE ENDOGENOUS AMINO ACIDS

There are several methods of determining endogenous amino acids which include feeding a nitrogen-free diet, and using fasted animals, the regression technique, feeding a highly digestible purified diet, and the peptide alimentation technique. In the first method, a nitrogen-free diet is fed to animals and the excreta or ileal digesta is then analyzed for amino acids. This is considered to be a classical technique to determine levels of endogenous amino acids because a diet containing no amino acids can still provide adequate stimulus for the digestive tract to secrete endogenous proteins (Ravindran and Bryden, 1999). In pigs, however, it has been reported that a nitrogen-free diet can lead to an overestimation of ileal proline and glycine flows and may lead to an overall underestimation of basal endogenous losses (Leterme et al., 1996; Stein et al., 2007). Excreta from fasted animals estimates the flow of amino acid without the presence of feed in the gut and is routinely used in the precision-fed cecectomized rooster assay (Parsons, 2002). However, the validity of these technique has been questioned

because without dietary protein, the metabolism of the animal is altered and can no longer be considered to be physiologically normal (Low, 1990). Fasted animals are in a negative nitrogen balance and the rate of whole-body protein synthesis can fall causing an increase of endogenous protein to enter the gut (Ravindran and Bryden, 1999).

One way to overcome the potential errors of using a fasted bird or feeding a nitrogen-free diet is to use the regression technique. This method determines endogenous amino acid flow in excreta or ileal digesta by feeding animals graded levels of a dietary protein and using regression analysis to extrapolate and calculate endogenous output at zero intake. The increased excretion of amino acids, which may be from undigested feed and endogenous proteins, are assumed to be directly proportional to the increased intake (Adedokun et al., 2007; Angkananporn et al., 1996; Siriwian et al., 1993). One major assumption of this method is that there are no changes in the amount of basal endogenous amino acids secreted and that the increase of ileal amino acid flow is attributed entirely to increases in undigested food proteins (Ravindran and Bryden, 1999). Other constraints are the assumption that the response is linear and the method may yield high standard errors (Moughan, 2003). Endogenous amino acids estimated using the regression method have been reported to be lower than (Angakanaporn et al., 1996), similar (Adedokun et al., 2007; Jansman et al., 2002) or higher than values obtained from feeding a nitrogen-free diet (Siriwan et al., 1993).

Another potential method to overcome the physiological abnormalities of feeding a nitrogen-free diet or using fasted animals is to feed diets that contain a highly digestible protein source (Chung and Baker, 1996; Ravindran and Hendriks, 2004). An example of a highly digestible protein is casein (intact or enzyme hydrolyzed, EHC). Results

reported in swine have not been consistent. Leibholz (1982) reported similar endogenous amino acid values for both nitrogen-free and casein diets. Fuller and Cadenhead (1991) reported lower ileal endogenous nitrogen losses in pigs fed a diet with added casein and crystalline amino acids in comparison to pigs fed only the nitrogen-free diet. However, Chung and Baker (1992) reported higher endogenous amino acids and nitrogen flow in pigs fed intact casein and crystalline amino acid in comparison to pigs fed a nitrogen-free diet. In poultry, 5, 15, and 21 d-old broiler chicks and turkey poults fed a highly digestible protein containing casein had an increased endogenous amino acid flow relative to birds fed a nitrogen-free diet (Adedokun et al., 2007). The increased endogenous amino acids from feeding highly digestible protein may be due to increased stimulation of digestive secretions or to incomplete digestion of protein in casein.

Another concern when feeding an intact highly digestible protein is the assumption regarding the complete digestibility of the ingested protein. This can be overcome by partially hydrolyzing the proteins and by separating endogenous proteins from the undigested dietary peptides (Butts et al., 1993). This peptide alimentation method may yield better estimates of basal ileal endogenous amino acids. This method involves feeding the animal peptides from EHC and then ultrafiltering the ileal digesta (Moughan et al., 1990). This technique is based on the differences in physical properties of nitrogenous fractions in the digesta and separation of the dietary free amino acids and small peptides from the large undigested endogenous proteins in ileal digesta based on molecular weights. By feeding a semi-synthetic diet of EHC as the sole source of protein, ileal digesta are then collected and the nitrogenous fractions are separated using centrifugation and ultrafiltration (Ravindran and Bryden, 1999). Butts et al. (1993)

reported that this method may yield lower estimates of basal endogenous ileal amino acid flow than feeding synthetic amino acids or intact highly digested proteins.

TRUE AND STANDARDIZED DIGESTIBILITY

Due to potentially confounding effects of endogenous amino acids discussed above, apparent digestibility values are usually corrected for endogenous amino acid losses. When this occurs the values are referred to as true or standardized digestibility. True and standardized amino acid digestibility values are often used synonymously but refer to two different concepts. Both digestibility values correct for endogenous amino acid losses but differ in the types of endogenous amino acid loss measured (Adedokun, 2007). True digestibility corrects for both the basal and diet induced endogenous amino acid flow. The separation of endogenous amino acids from undigested dietary amino acids at the terminal ileum after feeding a specific protein has become possible due to recent developments in isotope-dilution and homoarginine methods (Bryden et al., 1996; Schulze et al., 1995). While true digestibility may be of interest, the procedures to measure diet specific endogenous losses are labor intensive and require expensive, specialized equipment (Siriwan et al., 1994; Stein et al., 2007). Apparent digestibility values that are corrected for only basal endogenous amino acid losses using are considered to be standardized digestibilities (Lemme et al., 2004). The latter values are more frequently used to currently because they only correct for basal or diet independent endogenous amino acid contributions (Parsons, 2002; Ravindran and Bryden, 1999).

IN VITRO METHODS

Amino acid digestibility can be estimated using *in vitro* or *in vivo* methods. *In vitro* methods, like chemical, microbiological, and near infrared reflectance (NIR)

spectroscopy assays, are advantageous for their simplicity and rapid turnaround time. These methods are generally reproducible and require no animal use, which is favored by many institutions due to increasing pressure to reduce or cease animal use in research. *In vitro* methods can also give insight into the degree of heat damage of proteins, which can adversely affect the digestibility of amino acids, especially lysine. Lysine is second limiting in most practical poultry diets and its ε-amino group is highly susceptible to the Maillard reaction during heat treatment. *In vitro* assays, such as chemical assays, can estimate the amount of available amino acids; particularly lysine, however, they are not widely accepted because the values are not practical for commercial feed formulations (Ravindran and Bryden, 1999).

INDIRECT IN VIVO METHODS

The widely accepted methods of determining amino acid digestibility are the *in vivo* methods. *In vivo* methods can be categorized as either direct or indirect methods. The indirect methods of determining amino acid bioavailability include microbiological assays, insect assays, and plasma amino acid assays (Parsons, 2002; Sibbald, 1987). The indirect method of measuring blood plasma amino acids is based on the principle that the blood will transport any products of digestion and absorption (peptides and free amino acids) to tissues in the body (Ravindran and Bryden, 1999). However, the indirect measuring of plasma amino acids is based on the relationship between free amino acids and amino acid absorption. Plasma amino acids concentrations from starvation are used as a reference and compared to post-prandial plasma amino acids. This method is considered to be quick and convenient but is dependent on many factors such as nutritional status, age, and circadian rhythms (Low, 1990; Sibbald, 1987). Also, any

changes in plasma amino acids are hard to interpret and to quantitatively estimate changes in amino acid digestibility or availability. Due to these limitations, the plasma amino acid assay has not been widely used and accepted; however, this type of assay has been used for determining the limiting amino acids in poultry diets and to determine amino acid requirements (Fernandez-Figares et al., 1997). Therefore, it is most widely accepted to use direct *in vivo* methods.

DIRECT IN VIVO METHODS

GROWTH ASSAYS

There are two types of direct *in vivo* assays, growth and balance or digestibility assays. Growth assays are based on the principle that the amino acid in a protein or feedstuff will have the ability to provide a specific amino acid in supporting growth, a representation of protein accretion (Ravindran and Bryden, 1999). These growth assays usually involve the addition of graded levels of a specific amino acid or test feedstuff to an amino acid deficient diet. This method is called the slope-ratio method but if more than one test feedstuff is fed one can also use the standard curve method if only one level of the test feedstuff is fed. Bioavailability is calculated by regression analysis and from the ratio of the slopes of the growth lines for the test feedstuff and amino acid of interest (Parsons, 2002; Sasse and Baker, 1973). The measurement of the growth response to the dietary amino acid levels is favorable because this includes digestion, absorption, and utilization of the amino acid. However, these types of assays are expensive, time consuming, can only measure one amino acid at a time, and require expensive purified or semi-purified diets (Ravindran and Bryden, 1999).

DIGESTIBILITY ASSAYS

The more favored method for measuring amino acid availability is the digestibility bioassay. This assay is widely favored due to its ability to measure all amino acids in one assay and having values that are directly applicable to the animal being studied. Digestibility assays for poultry can be conducted by collecting either excreta (feces and urine) voided from the animal or by collecting digesta from the ileum. Excreta assays are based on the principle of measuring amino acids that are voided in the excreta, which are then subtracted from dietary amino acids. Even though feces and urine are collected together, it has been shown that the amino acid content of urine is small and will have little effect on amino acid digestibility values (Ravindran and Bryden, 1999; Terpstra, 1978). Adult roosters are preferred animal subjects because they do not lay eggs, which, when broken, can contaminate the excreta sample. The excreta method also has advantages that it is a fairly simple assay to conduct and a large number of animals may be utilized without euthanizing or making surgical modifications. The most common excreta method is the precision-fed rooster assay.

PRECISION-FED CECECTOMIZED ROOSTER ASSAY

Many of the previous studies on digestible amino acids for poultry have been based on the excreta assay. Sibbald (1976) developed a rapid feeding assay which involved precision-feeding adult roosters to evaluate metabolizable energy and digestible amino acids of feedstuffs. This assay has often been used to determine amino acid digestibility and is commonly called the precision-fed rooster assay. After fasting birds prior to feeding and then precision-feeding a known quantity of sample, excreta are then quantitatively collected over a period of 48 hours and analyzed for amino acids.

Endogenous amino acid losses are calculated by either collecting excreta from fasted roosters or precision-feeding a protein-free diet (Ravindran and Bryden, 1999). Correcting for endogenous losses allows for a true or standardized digestibility coefficient to be determined. But, due to the microbial fermentation that occurs in the avian ceca, there has been some debate on whether intact roosters and excreta assays can correctly estimate amino acid digestibility (Bryden et al., 1990).

The main site of microbial fermentation of avian species is the ceca. The ceca are two blind pouches located near the terminal ileum and colon. The major microbial activity in the poultry ceca is fermentative with Gram positive anaerobic cocci as the predominant type with a large population of uric acid degrading bacteria also being found in the ceca (McNab, 1973). For amino acid utilization, this is the site where the majority of the microorganisms in the poultry intestine may degrade any undigested dietary amino acids (Mead, 1989; Parsons, 1986). It has been reported that microbial protein may contribute approximately 25% of the total excreta protein (Parsons et al., 1982). This microbial modification of amino acids as well as the microbial protein contribution in feces may change final excreta analysis values, which can influence the digestibility values calculated from a total excreta analysis (Ravindran and Bryden, 1999). In order to overcome this obstacle, it has been proposed to surgically remove the ceca in order to more accurately evaluate amino acid digestibility since the primary site of microbial fermentation will be removed. Cecectomy is considered simpler and more rapid in comparison to other surgical procedures such as ilea1 cannulation (Parsons, 2002). The microbial capacity and size of the avian ceca is considerably less than that of most mammals. Amino acid digestibilities determined in cecectomized roosters are often

different than those determined in conventional or intact roosters. Parsons (1986) reported that standardized amino acid digestibility values of cecectomized roosters were more similar to bioavailability growth assay values in chicks than were standardized digestibility values from intact roosters. The effect of cecectomy had no effect on amino acid digestibility for cereal grains but yielded small differences in oilseed meals (Green et al., 1987). Green and Kiener (1989) reported no differences in true amino acid digestibility in soybean meal and sunflower meal between conventional and cecectomized roosters but a significant decrease in amino acid digestibility of animal by-products for cecectomized birds. More recently, amino acid digestibility values for animal protein supplements like feather meals and meat and bone meals were reported to be generally lower in cecectomized roosters when compared to conventional roosters (Han and Parsons, 1991; Parsons, et al., 1997).

The precision-fed cecectomized rooster has been the most frequently used amino acid digestibility assay for many reasons. The assay has a reduced time and expense in comparison to plasma amino acid and growth assays. Each bird is only crop intubated 30 g of feed, so only a small amount of feed is required. There is no need for a digesta marker and amino acid digestibility values can be obtained for any feedstuff, regardless of palatability due to the crop intubation of the assay. This type of assay can be used routinely to evaluate amino acid digestibility of a large number of samples (Parsons, 2002). Another important advantage of the precision-fed cecectomized rooster assay is the ability to obtain a cysteine digestibility value. Cysteine digestibility values cannot be easily achieved from a growth assay. Two assays would need to be conducted, one to determine methionine and cysteine digestibility and a second to determine just

methionine digestibility. Cysteine digestibility is then determined by calculating the difference (Parsons, 1986).

However, despite all the advantages of the precision-fed rooster assay, there are still some criticisms of this type of assay. Due to the nature of the crop intubation, this assay does not mimic natural feeding behaviors. Adult birds are primarily used so it has been suggested that amino acid digestibility values may not be applicable to younger animals. This may not be a problem with most ingredients, but it may overestimate amino acid digestibilities for ingredients like wheat and barley (Parsons, 2002). Low protein ingredients, like grains, can yield highly variable amino acid digestibilities, which result because of very low amino acid intake (Stein et al., 1999). More recently, with increasing animal welfare concerns, the cecectomy surgical procedure necessary for this assay is becoming increasingly more difficult to get approval by Institutional Animal Care and Use Committees and is leading researchers to explore new options for evaluating amino acids in feedstuffs. Despite all these obstacles, the precision-fed cecectomized rooster assay has been widely used as a method for evaluating amino acid digestibility.

ILEAL DIGESTIBILITY ASSAYS

In response to some of the criticisms of excreta assays, Payne et al. (1968) suggested using an ileal digesta method to measure amino acid digestibility more accurately. This method is based on the principle that contents collected in the ileum may be a more accurate measure of digestibility since there will be very little microbial alterations in the distal small intestine in comparison to excreta collection. Ileal amino acid digestibility can be evaluated either by inserting a cannula in the terminal ileum or

slaughtering birds and collecting digesta from the distal small intestine. The most commonly used technique is the slaughter technique where the contents of the entire ileal region are collected (Adedokun et al., 2007, 2008; Ravindran and Bryden, 1999). However, because the slaughter technique involves sacrificing many birds, some researchers have suggested using ileal cannulation. It was reported that apparent ileal amino acid digestibility values in ileal cannulated chickens were significantly lower for all amino acids in comparison to amino acid digestibility values determined using the ileal slaughter method (Johns et al., 1986). Ileal cannulation in birds is a difficult and time consuming procedure (Ravindran and Bryden, 1999; Tanksley et al., 1981). Ileal cannulation is also limited by variable digesta flow through the cannula and rejection of the cannula by the animal (Parsons, 2002). Therefore, the most commonly used method is to feed chickens and then euthanize them to collect contents of the ileum. Birds are fed an experimental diet with an appropriate marker over a period of several days or weeks (Adedokun et al., 2008; Parsons, 2002). Upon completion of the experimental period, the birds are euthanized and ileal digesta are collected. Digesta are generally collected for the entire ileal region between the Meckel's diverticulum and the ileo-cecal junction; however, it has been suggested that collection from only the last 15-20 cm of the small intestine may be preferred (Kadim and Moughan, 1997).

DIGESTIBILITY MARKERS

The use of the ileal digesta technique requires an indigestible marker to relate the amino acid contents in the ileum to those in the diet. The most effective markers will be inert materials that are not digested or absorbed within the gastrointestinal tract and have no effect on the digestive system. Amino acid digestibility is determined by the ratio of the

concentration of the marker in the diet to the concentration in the ileal digesta or feces. For amino acid digestibility assays, the most commonly used markers are chromic oxide (Cr_2O_3), acid insoluble ash (AIA), and titanium dioxide (TiO_2).

One of the most commonly used indigestible markers is chromic oxide. It has a molecular weight of 152.02 and is one of several chromium compounds (Kotb and Luckey, 1972). Chromic oxide is effective as an indigestible marker because it can be added at very low (0.25-0.50%) inclusion rate. Chromic oxide is generally light green to dark green in color and is non-toxic to animals (Kotb and Luckey, 1972). It is desirable because it is well incorporated into diets and can be delivered by voluntary feed consumption or by gavage and is carried in the solid phase of digesta, which makes it acceptable for ileal collection. Kotb and Luckey (1972) reported that apparent digestibility data using chromic oxide were not significantly different from data using the quantitative method for a number of species, including poultry, indicating the validity of the chromic oxide method. More recently, however, Oberleas et al. (1990) criticized the use of chromic oxide because it may be more readily carried in the fluid phase of digesta rather than the solid phase of the digesta. It has also been reported that there is high variability and low repeatability for the analytical assay for chromic oxide (Sales and Janssens, 2003) but this may be due to inadequate sample being provided. In comparison to other methods like AIA and total collection, chromic oxide was reported to be less suitable for measuring apparent metabolizable energy (AME) due to an uneven flow rate (Oberleas et al., 1990; Scott and Boldaji, 1997). An advantage of the chromic oxide method is that only a small amount of sample is required to run chemical analysis (Scott and Boldaji, 1997).

Another commonly used indigestible marker is acid insoluble ash (AIA). The principle behind this method is that the sample, usually Celite® (diatomaceous earth) or silica is added to a diet or feed and because it is not absorbed in the gastrointestinal tract, it can be recovered in the feces. Through acid digestion, the Celite® will precipitate into a salt (NHCl) to form a residue, which can be easily measured in feces, ileal digesta, and feed. The primary disadvantage of using AIA is that its analysis is based on a gravimetric measurement which necessitates a large quantity of sample, approximately 3 g, for accurate analysis (Scott and Boldaji, 1997). Scott and Hall (1998) reported that using the AIA method to determine AME and nitrogen retention was more accurate. Tillman and Waldroup (1988) reported that AIA may yield a better measurement for AME because of its ad libitum feeding and shorter collection period, compared to the total collection method, which requires feed withholding periods. Sales and Janssens (2003) reported higher digestibility values using AIA methods when compared to the quantitative excreta collection method, which may have been caused by variation in AIA analysis. When hydrochloric acid treatment occurs after ashing of the samples, it can overestimate digestibility in avian species (Sales and Janssens, 2003).

More recently, titanium dioxide, another inert marker, has been used in poultry nutrition studies. It is a white powder that was first proposed as a digestibility marker by Askew (1931). Kotb and Luckey (1972) reported that titanium dioxide was a better marker for estimating quantitative intestinal absorption than ferric oxide, which had been the favored marker at that time. Titanium dioxide also has a similar rate of passage as chromic oxide, which makes it an ideal comparison marker. Njaa (1961) found that titanium dioxide was useful in the study of protein digestion in rats since it can be readily

estimated in the Kjeldahl N digestion analysis of feed and feces. The analysis of titanium dioxide is an accurate and simple colorimetric analysis. Currently, only one poultry nutrition study has evaluated the recovery of the marker in excreta (Sales and Janssens, 2003). Peddie et al., (1982) reported less variation in dry matter digestibility when using titanium dioxide than with the total excreta collection method. Either chromic oxide or titanium dioxide are usually used in ileal digestibility studies for poultry because much more sample is needed for analysis.

COMPARISON OF METHOD, AGE, STRAIN, AND SEX ON AMINO ACID DIGESTIBILITY VALUES

While many studies have evaluated the individual merits of each amino acid digestibility assay, only a few studies have compared multiple amino acid digestibility methods. Ravindran and Bryden (1999) reported that ileal amino acid digestibility values in some feed ingredients were similar to corresponding excreta values, but ileal values were significantly lower or higher in other ingredients when compared to excreta amino acid values. Garcia et al. (2007) reported that the standardized ileal amino acid digestibility values for chicks were significantly lower in comparison to the cecectomized rooster assay amino acid values for some ingredients and attributed these differences to age or methodology differences. Adedokun et al. (2009) compared amino acid digestibility in cecectomized rooster with ileal digestibilities in laying hens and broilers. Amino acid digestibilities were not significantly different between broilers and roosters in three (corn, light and dark distiller's dried grains with solubles; DDGS) of the six feed ingredients evaluated. For the other three feed ingredients (canola meal, soybean meal, and meat and bone meal) there were no significant differences between broilers and roosters. In addition, the ileal amino acid digestibility values were significantly greater in

laying hens than broilers for corn and meat and bone meal but there were no differences for the other feed ingredients evaluated.

FORMULATION OF DIETS ON A DIGESTIBLE AMINO ACID BASIS

Poultry diets have generally been formulated on a crude protein or total amino acid basis. More recently, it has been suggested that formulation of diets on a digestible amino acid basis may be more advantageous. Formulation of diets on a digestible amino acid basis may decrease feed costs, lower feed safety margins, and decrease nitrogen excretion into the environment (Applegate et al., 2008). Several studies have shown the advantages of formulating on a digestible amino acid basis when compared to a total amino acid basis. Rostagno et al. (1995) reported that formulating broiler diets on digestible amino acids gives a better prediction of dietary protein quality and bird performance than total amino acids. When broilers were fed a diet with low digestible amino acids from various by-products; body weight, feed efficiency, and breast meat yield were significantly reduced when compared to birds fed a diet formulated with high digestible amino acids (Rostagno et al., 1995). Fernandez et al. (1995) evaluated dietary formulation on a total versus a digestible amino acid basis for diets containing cottonseed meal and showed that chicks fed diets containing as much as 20% cottonseed meal formulated on digestible amino acids resulted in growth and feed efficiency similar to a corn-soybean meal diet. Wang and Parsons (1998) showed similar results with meat and bone meal samples; chicks fed a diet formulated on total amino acids resulted in less growth and feed efficiency when compared with chicks fed diets formulated on a digestible amino acid basis. Douglas and Parsons (1999) reported that chicks fed a diet formulated with spent-hen meal based on digestible amino acids had increased growth

performance when compared with diets formulated on total amino acids. More recently, Khaksar and Golian (2009) evaluated diets based on ileal digestible amino acids versus total amino acids in broiler performance using a corn-soybean meal diet. Broilers fed diets formulated on digestible amino acids had increased weight gain, breast yield, lower feed to gain ratios and abdominal fat pad when compared to birds fed diets formulated on total amino acids (Khaksar and Golia, 2009). These studies all indicate the advantages of formulating diets on a digestible amino acid basis.

SUMMARY AND OBJECTIVES

Formulation of diets based on digestible amino acids is preferable to formulation based on total amino acids. The most used and accepted methods of determining digestible amino acids in poultry are the precision-fed cecectomized rooster assay and the standardized ileal chick assay. There have only been a few studies comparing these methods to determine if they yield similar digestibility values. Both Garcia et al. (2007) and Adedokun et al. (2009) evaluated and compared the cecectomized rooster and ileal chick assays and reported that the rooster assay yielded higher values than the chick assays for some ingredients but not others. The results of these two methods suggest that the amino acid digestibility values from the rooster assay may be greater than those from the chick assay, but results are not conclusive at present. The first objective of this dissertation was to more extensively determine and compare amino acid digestibility values between the precision-fed cecectomized rooster assay and the ileal chick assay. The second objective was to develop a new precision-fed ileal chick assay that would be rapid, convenient, and require only a small amount of feed sample. The third and final objective was to determine amino acid digestibility for several feed ingredients using the

new precision-feeding ileal chick assay and to compare those values to those obtained from the cecectomized rooster and standardized ileal chick assays.

In poultry nutrition, it is imperative to determine amino acid digestibility of feedstuffs. Formulation of diets based on digestible amino acid values is economically viable and has been shown to produce more efficient birds. The most accepted methods of determining amino acid digestibility in poultry are the precision-fed cecectomized rooster assay and the standardized ileal chick assay. The digestibility of feedstuffs using these methods has been widely studied; however, there have only been a few studies comparing these methods. The objective of this dissertation is to determine and compare the amino acid digestibility of feedstuffs that are commonly used in the poultry. This objective will be carried out by using various methods and developing a new precision-fed ileal chick assay.

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Chapter 2

COMPARISON OF AMINO ACID DIGESTIBILITY OF CORN, CORN DISTILLER'S DRIED GRAINS WITH SOLUBLES (DDGS), MEAT AND BONE MEAL (MBM), AND POULTRY-BY-PRODUCT MEAL (PBPM) DETERMINED WITH THE PRECISION-FED CECECTOMIZED ROOSTER ASSAY AND THE STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY CHICK ASSAY

ABSTRACT

The objective of this study was to evaluate and compare the amino acid digestibility of several feedstuffs using two commonly accepted methods; the precision-fed cecectomized rooster assay (PFR) and the standardized ileal amino acid chick assay (SID). To carry out these objectives, 17 different feedstuffs were obtained. These samples included six corn, six corn distiller's dried grains with or without solubles (DDGS/DDG), one wet distiller's grains, one condensed solubles, two meat and bone meal (MBM) and a poultry-by-product meal. The wet distiller's grains and condensed solubles were only evaluated in roosters. Standardized amino acid digestibility was found to vary among the feed ingredients and among samples of the same ingredient. For corn, there were generally no differences in amino acid digestibility between the two methods. When differences did occur, there was no consistent pattern among the individual amino acids and methods. Standardized amino acid digestibility was not different between the two methods for the four DDGS samples; however, the PFR did yield greater digestibilities for a high protein DDG and a conventionally processed DDGS. The PFR yielded greater amino acid digestibility values than the SID for several amino acids in one MBM and the poultry-by-product meal, but it yielded lower digestibility values for the other MBM. Overall, there were no consistent differences between methods for amino acid digestibility values.

INTRODUCTION

Many feed ingredients used in poultry production can be variable in nutrient content from batch to batch. One of the most important nutrients in diet formulations is protein or amino acids. Three commonly used feedstuffs used in poultry diets are corn, corn distiller's dried grains with or without solubles (DDGS or DDG; DDG/S) and animal by-product meals. Previous research has shown that these ingredients can vary in protein content and protein quality. The crude protein and amino acids in corn can be affected by genetics, location, soil type and fertility, rainfall and other environmental factors. For example, Cromwell et al. (1999) reported a significant difference in the crude protein of corn grown in different states, with a difference that ranged from 7.3 to 9.0% crude protein. With the advent of new biotechnologies, new transgenic strains of corn are being developed. Most of these new transgenic strains have added herbicide tolerance and pest resistance (Taylor et al., 2003). In a review of 23 research experiments, Clark and Ipharraguerre (2001) reported that transgenic corn and soybean meals with herbicide tolerance and pest resistance were reported to have no differences in nutrient quality and value in comparison to conventional corn and soybeans. However as companies continue to stack multiple traits for herbicide tolerance and herbicide resistance in corn, this may eventually result in differences in nutrient variability between transgenic and conventional corn and therefore, these corns should be continuously evaluated for their nutrient value, particularly amino acid content and digestibility.

Corn distiller's dried grains with or without solubles (DDGS or DDG) is a co-product of the ethanol industry. One of the main factors limiting poultry producers in

utilizing DDGS is the variability in nutritional value (Gibson and Karges, 2006). The protein content has been determined to be highly variable among batches of DDGS. A major reason for this high variability may be due to the variation in the proportions of wet distiller's grains (WDG) and the solubles in the final DDGS. The mixing of WDG and solubles in ethanol plants is not highly regulated and may lead to high variability in the final protein concentration of the DDGS (Belyea et al., 1998). Another big concern involving the use of DDGS is related to the variability in lysine digestibility. Due to the high moisture content of the WDG and solubles, they must be dried to extend their shelf life and to produce a product that can be easily shipped and used in commercial feeding systems. Therefore, in a conventional dry grind plant, the wet grains are dried to reduce moisture content from 63.7 to 9.9%, which requires high temperatures (Kwiatkowski et al., 2006). Due to these high temperatures and length of drying required, there is a possibility that the proteins in the DDGS may become damaged, thus creating a loss of available amino acids, mainly lysine, due to the Maillard reaction between lysine and reducing carbohydrates (Spiehs et al., 2002).

Animal-by-product meals, such as meat and bone meal (MBM), can also vary in protein quality. This variation can result mainly due to differences in the origin of the animal protein as well as differences in processing conditions (Parsons et al., 1997; Ravindran and Bryden, 1999). Parsons et al. (1997) reported substantial variability among 16 different MBM samples. The crude protein ranged from 47.8 to 57.8% and lysine bioavailiability ranged from 43 to 89% (Parsons et al., 1997). Adedokun et al. (2007b) also reported variable crude protein and apparent ileal digestibility values for 4

different MBM samples and attributed these differences to processing and composition of the raw materials prior to rendering.

The two animal assays that have historically been used most often to determine digestibility or bioavailability of amino acids in feed ingredients for poultry are the slope-ratio growth assays (bioavailability) and the balance assays (digestibility). The balance assays are used much more frequently than the growth assays because they are much faster, less expensive and all amino acids can be evaluated in one assay. The balance or digestibility assay that has been most frequently used for poultry during the last 20 years is the precision-fed cecectomized rooster assay (Parsons, 2002). More recently, there has been increased use of the newer standardized ileal amino acid digestibility chick assay (Adedokun et al., 2008; Ravindran and Bryden, 1999). In a recent study by Garcia et al., (2007) wherein a few ingredients were evaluated, it was found that the cecectomized rooster and the ileal chick assay sometimes yielded significantly different amino acid digestibility values for some ingredients, with the rooster values being greater than the chick values. In another study (Adedokun et al., 2009), standardized amino acid digestibility in cecectomized roosters for several feedstuffs was compared to the ileal digestibility in broilers and laying hens. In the latter study, roosters yielded significantly higher amino acid digestibilities for MBM in comparison with broiler chicks, while corn was not significantly different between the two assays. In addition, one DDGS sample was reported to have greater digestibility in roosters when compared to broilers, while another dark DDGS sample did not have any significant differences between methods (Adedokun et al., 2009).

The objective of this study was to determine and compare the amino acid digestibility of 17 different feedstuffs and samples between the precision-fed cecectomized rooster assay (PFR) and the standardized ileal amino acid digestibility chick assay (SID).

MATERIALS AND METHODS

Feed Sample Analysis

Six corn samples, eight DDGS or DDG samples, two MBM samples, and a poultry by-product meal were obtained. Strain, processing, and other sample descriptions are presented in Table 2.1 for all samples. The wet distiller's grains and condensed solubles were freeze-dried and ground. All feedstuffs were analyzed for N and amino acids (AOAC International, 2000: method 99n/a3, 982.30 E (a, b, c) at the Experiment Station Chemical Laboratories, University of Missouri-Columbia).

Standardized Ileal Amino Acid Chick Assay

All animal care, handling, and euthanasia were approved by the Purdue University Animal Care and Use Committee. This assay was conducted using the procedures described by Adedokun et al. (2008). Male Ross 308 broiler chicks were obtained at 1 d of age from a commercial hatchery, weighed individually, and fed a nutritionally complete starter diet until d 16 before they were placed on the experimental diets. At that time, birds were randomized to cages with 8 birds per cage, 6 replicate pens per experimental diet. The birds were then fed the 15 experimental diets until 21 d of age. The wet distiller's grains and condensed distiller solubles were not fed to the broiler chicks due to insufficient amounts of sample. On d 21, birds were killed by CO_2 asphyxiation and ileal digesta were collected. The contents of the ileum were considered

to be the part of the small intestine from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction. The ileal digesta from birds were pooled, frozen, and stored at -20ºC until they were processed. For the rooster assay, the excreta were also frozen and stored at -20ºC until processing. All ileal samples were freeze-dried, ground by using a mortar and pestle and then analyzed for amino acids as described earlier for feed analysis.

Diet Formulation

The semi-purified experimental diets (Adedokun et al., 2007a) were formulated to contain approximately 20% crude protein (CP) (with the exception of the corn diets, which was approximately 7% CP), with each of the feedstuffs supplying the only source of CP in the diets. All the feedstuffs were analyzed for CP before diet formulation. Chromic oxide was added to the diet as an indigestible marker at 0.30% of the diet, with all diets being fed in mash form.

Precision-fed Cecectomized Rooster Assay

A precision-fed rooster assay utilizing cecectomized Single Comb White Leghorn roosters was conducted (Parsons, 1985). All animal housing, handling, surgical, and euthanasia procedures were approved by the University of Illinois Animal Care and Use Committee. After 24 hours of feed withdrawal, four cecectomized roosters (approximately 38 weeks old) were tube-fed approximately 30 grams of each of the 17 feed samples. Excreta were then quantitatively collected for 48 hours, freeze-dried, weighed, ground, and analyzed for amino acids as described earlier. Endogenous corrections for amino acids were made using five roosters that had been fasted for 48 hours.

Calculations

Amino acid digestibility was calculated for the SID chick assay were calculated using the following formulas by Moughan et al. (1992). The apparent ileal amino acid digestibility coefficients were standardized using the ileal amino acid values from 21 d old broiler chicks fed a nitrogen-free diet (Adedokun et al., 2007a).

APPARENT ILEAL AMINO ACID DIGESTIBILITY =

[1 − (chromium in diet/chromium in ileal digesta) × (amino acid in digesta/amino acid in diet)]

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =

Apparent digestibility + [(IEAA flow, g/kg of DMI)/(amino acid content of the diet, g/kg of DM)] × 100.

For the rooster assay, standardized amino acid digestibility value was calculated with the following formula. The amino acids were standardized by using an endogenous correction based on amino acid excretion by fasted roosters.

STANDARDIZED AMINO ACID DIGESTIBILITY, %=

[(Amino acid fed (mg) − Amino acid excreted (mg) + Endogenous amino acid excreted (mg))/ Amino acid fed (mg)] × 100.

STATISTICAL ANALYSIS

All data from both assays were analyzed using PROC GLM (SAS Institute, 1990) for a completely randomized design. Differences between treatment means were separated using the PDIFF option in the least-square means (LSMEANS) procedure of GLM. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The total amino acid concentration of the six corn samples is presented in Table 2.2. There was not much variation in amino acid concentration among the six samples of corn. These values are also in agreement with previously published data (NRC, 1994; Parsons et al., 1998). The standardized amino acid digestibility coefficients for the six corn samples determined in the PFR and the SID are presented in Table 2.3. There were generally no consistent differences in amino acid digestibility between the two digestibility methods. Values for the corn Samples 1 and 4 were similar between digestibility methods. Several values for corn Samples 2 and 6 were higher for the SID and several values for Samples 3 and 5 were higher for the PFR. Interestingly, the SID yielded consistently higher His digestibilities for all six corn samples. The reason for the latter results is unknown.

The total amino acid concentrations for the six DDGS samples are presented in Table 2.4. There was variation among the different samples in total amino acid concentration with the greatest variation being the much higher values for the high protein DDG Sample 5. The standardized amino acid digestibility coefficients for the six DDGS samples determined in the PFR and SID are presented in Table 2.5. The standardized digestibility values for both assays were in general agreement with previously published values (Adedokun et al., 2008; Batal et al., 2006). For DDGS Samples 1, 2, 3, and 4, there were no significant differences between the two methods for most of the amino acids. However, the SID did yield significantly greater digestibility values for Met and Cys for some of the samples. These four DDGS samples were produced in a newer fuel ethanol plant and varied in the ratio of wet distiller's grains to

condensed solubles and the addition of recycled DDGS back into the dryers (Table 2.1) (Kingsley et al., 2010). In contrast to the first four DDGS samples, the PFR yielded higher (P<0.05) digestibility values for almost all amino acids in Samples 5 and 6. Sample 5 was a high protein DDG and Sample 6 was a conventional DDGS. The high protein DDG was obtained by fractionating the corn kernel prior to fermentation to remove pericarp fiber and fermenting only the endosperm portion of the corn kernel (Applegate et al., 2009). The high amino acid digestibility obtained with the roosters may have been partially due to the reduced fiber in the high protein DDG. Kim et al. (2008) also reported that a similar high protein DDG had higher amino acid digestibility than conventional DDGS when evaluated in a PFR. The nutritional composition and standardized amino acid digestibility of the WDG and CDS from the PFR are presented in Table 2.6. As mentioned earlier, due to the small amount of sample obtained, these samples were only fed to cecectomized roosters. These two samples were obtained from the processing stream prior to producing DDGS Samples 1-4. The CDS had lower total amino acid concentrations than the WDG, which is in agreement with previously published research (Martinez-Amezcua et al., 2007).

The total amino acid concentrations of the two MBM and poultry by-product meal are presented in Table 2.7. MBM 1 had increased amino acid content in comparison with MBM 2. Both MBM samples consisted of mixed raw materials and were rendered in a continuous horizontal cooker and had approximately the same range in crude protein for the raw materials (50-54% and 49-55% for MBM 1 and 2, respectively). However, the MBM 1 was analyzed to contain approximately 25% ash while MBM 2 had an approximate 29% ash content. Thus, the higher protein content of MBM 1 was probably

due to the lower ash content. Likewise, total amino acid concentrations were consistently lower for MBM 2, which may be due to the higher ash content. The standardized amino acid digestibility values determined in the PFR and SID are presented in Table 2.8. For MBM 1, the PFR yielded significantly ($P<0.05$) or numerically higher amino acid digestibility values than the SID. Conversely, for MBM 2, the chick assay yielded higher amino acid digestibility values than the rooster assay for several amino acids. The total amino acid content of poultry by-product meal was in agreement with previously published research (Johnson et al., 1998; NRC, 1994). Standardized amino acid digestibility values determined by the SID were significantly lower ($P<0.05$) than values determined by the PFR.

In conclusion, the results of this study support those of earlier research that has shown that amino acid digestibility varies among feed ingredients and among samples of the same ingredient. There were no consistent differences between digestibility methods for amino acid digestibility values determined with the PFR and SID. Overall, for the 15 feed ingredients evaluated in both assays, six ingredients had digestibility values that were similar between methods, six had values that were greater for the PFR than the SID, and three had values that were greater for the SID than the PFR.

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Table 2.1 Sample number and description of sample

Sample Number	Description
Corn 1	High extractable starch variety - Purdue University harvest 2007
Corn 2	Transgenic corn triple-stacked trait variety - Purdue University harvest 2007
Corn 3	Transgenic corn, Round-up Ready variety - Purdue University harvest 2007
Corn 4	Low phytate high oil - Purdue University harvest 2007
Corn 5	Mixed variety – Purdue University harvest 2006
Corn 6	Mixed variety – Purdue University harvest 2007
DDGS 1[1]	7.39% CDS added to increase CDS to WDG ratio with recycled DDGS added back to dryers-Indiana plant
DDGS 2[1]	3.69% CDS added to increase CDS to WDG ratio with recycled DDGS added back to dryers-Indiana plant
DDGS 3[1]	No additional CDS to the CDS to WDG ratio with recycled DDGS added to dryers-Indiana plant
DDGS 4[1]	3.69% CDS added to increase CDS to WDG ratio, with no recycled DDGS added to the dryers-Indiana plant
DDG 5	High protein DDG with no solubles added –Southern IL pilot plant
DDGS 6	DDGS - plant site unknown
DDGS 7[1,2]	WDG prior to processing schemes-Indiana plant
DDGS 8[1.2]	CDS prior to processing schemes-Indiana plant
MBM 1	50-54% CP, 24-26% ash, continuous horizontal cooker, mixed raw material-plant site unknown
MBM 2	49-55% CP, 26-32% ash, continuous horizontal cooker, mixed raw material-plant site unknown
PBPM	Obtained from Alabama

DDG/S=Distiller's dried grains without/with solubles; CDS=Condensed distiller's solubles; WDG=Wet distiller's grains; MBM=Meat and bone meal; PBPM=Poultry by-product meal

[1] Further processing details in Kingsly et al. (2010).

[2] DDGS 7 and 8 were not fed through the standardized ileal chick assay.

Table 2.2 Total amino acid concentrations (%) of the six corn samples, as-fed basis

	Corn Sample number					
Amino acid	1	2	3	4	5	6
Aspartic acid	0.62	0.53	0.53	0.59	0.53	0.68
Threonine	0.34	0.28	0.30	0.30	0.27	0.33
Serine	0.40	0.36	0.39	0.38	0.35	0.42
Glutamic acid	1.67	1.46	1.45	1.54	1.35	1.63
Proline	0.71	0.61	0.61	0.62	0.58	0.68
Glycine	0.39	0.35	0.34	0.35	0.32	0.37
Alanine	0.70	0.58	0.58	0.58	0.53	0.60
Cysteine	0.17	0.15	0.15	0.16	0.14	0.16
Valine	0.46	0.38	0.36	0.39	0.34	0.40
Methionine	0.18	0.14	0.15	0.15	0.14	0.15
Isoleucine	0.37	0.32	0.30	0.33	0.27	0.34
Leucine	1.14	0.95	0.95	0.97	0.88	1.01
Tyrosine	0.36	0.32	0.32	0.32	0.30	0.34
Phenylalanine	0.47	0.40	0.39	0.41	0.37	0.43
Lysine	0.33	0.25	0.26	0.28	0.26	0.33
Histidine	0.26	0.22	0.22	0.23	0.21	0.24
Arginine	0.49	0.42	0.42	0.44	0.40	0.50
Tryptophan	0.06	0.09	0.08	0.09	0.11	0.11

Table 2.3. Standardized amino acid digestibility (%) for the six corn samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility chick assay (SID)

	Corn Sample number											
	1				2				3			
Amino acid	PFR[1]	SEM	SID[2]	SEM	PFR	SEM	SID	SEM	PFR	SEM	SID	SEM
Aspartic acid	91.9	2.0	90.5	0.8	82.1	7.9	89.9	1.4	89.5	4.4	88.9	1.2
Threonine	91.2	4.3	85.9	0.9	86.0	9.7	84.4	2.1	92.3[a]	3.1	83.6[b]	1.2
Serine	92.3	4.0	95.1	0.8	83.9	10.9	95.7	1.4	92.3	4.2	95.0	1.1
Glutamic acid	95.6	1.2	97.8	0.5	88.4[b]	3.8	98.2[a]	0.8	92.4[b]	2.5	97.7[a]	0.6
Proline	93.1	2.2	92.8	0.6	84.3	8.0	91.7	1.1	92.5	4.5	91.3	0.6
Alanine	95.3	1.4	93.5	0.8	89.2	5.0	93.1	0.9	94.4	2.7	92.6	0.6
Cysteine	93.0	4.9	89.8	0.6	84.3	13.2	88.1	1.7	97.7[a]	5.1	87.0[b]	1.1
Valine	89.5	3.2	90.7	0.5	80.4	9.4	89.8	1.5	84.8	3.2	88.0	1.1
Methionine	96.2	1.6	96.7	0.8	89.2	5.1	97.1	1.0	94.9	2.9	97.0	0.8
Isoleucine	95.1	1.8	95.4	0.7	87.4	6.5	95.5	1.2	94.3	3.5	94.8	1.0
Leucine	97.7[a]	1.2	93.8[b]	0.7	92.7	4.2	93.3	0.9	97.0[a]	2.0	93.1[b]	0.5
Tyrosine	92.6	1.9	91.3	0.6	83.8	5.6	91.3	1.1	90.0	3.5	90.5	0.8
Phenylalanine	95.7	1.9	92.4	0.6	88.8	5.5	92.1	1.0	94.5	3.0	91.5	0.7
Lysine	80.1	3.6	85.9	0.6	49.5[b]	10.5	84.4[a]	1.5	70.9[b]	3.9	84.2[a]	1.2
Histidine	88.2[b]	1.7	97.3[a]	1.1	71.1[b]	6.1	96.6[a]	1.1	79.0[b]	3.9	96.3[a]	0.7
Arginine	95.9	2.3	93.8	0.6	87.0	6.6	93.7	0.9	93.4	3.0	93.6	0.6
Tryptophan	95.1	2.4	n/a[3]	n/a	93.7	4.1	n/a	n/a	100.1	2.9	n/a	n/a

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).

[1]Mean of 4 roosters.

[2]Mean of 6 replicate pens of 8 chicks.

[3]n/a=not analyzed

Table 2.3. contd. Standardized amino acid digestibility (%) for the six corn samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility chick assay (SID)

	Corn Sample number											
	4				5				6			
Amino acid	PFR[1]	SEM	SID[2]	SEM	PFR	SEM	SID	SEM	PFR	SEM	SID	SEM
Aspartic acid	85.9	5.4	84.9	1.5	91.8[a]	2.0	78.5[b]	2.3	83.8	3.8	85.1	0.8
Threonine	87.8	6.3	78.9	1.7	93.5[a]	2.5	68.6[b]	3.0	84.9	4.4	80.0	1.2
Serine	86.8	7.3	92.2	0.9	94.8[a]	2.3	84.6[b]	1.8	84.8	4.5	90.6	0.9
Glutamic acid	92.0	3.7	95.5	0.8	93.6	1.0	91.7	1.5	89.1[b]	3.2	95.0[a]	0.3
Proline	87.4	4.8	88.6	1.0	93.1[a]	1.6	84.0[b]	1.2	81.7	4.5	88.9	0.9
Alanine	90.9	4.7	89.9	1.0	94.6[a]	1.1	85.4[b]	1.7	87.3	3.1	89.6	0.6
Cysteine	93.0	6.4	82.2	1.5	96.8[a]	2.8	74.7[b]	2.0	76.8	5.6	83.3	1.2
Valine	81.7	6.6	84.5	1.4	87.8[a]	2.3	75.5[b]	2.6	78.6	4.7	83.9	0.9
Methionine	91.9	5.2	94.4	1.2	95.5	1.9	89.0	2.2	87.1[b]	4.0	93.6[a]	0.7
Isoleucine	89.9	5.6	91.1	1.3	93.6[a]	1.9	84.6[b]	2.4	83.7	4.0	89.8	0.7
Leucine	94.1	4.0	90.5	0.9	97.1[a]	1.0	86.1[b]	1.6	91.1	2.2	90.1	0.4
Tyrosine	86.9	6.1	87.8	1.0	90.1[a]	2.5	82.3[b]	1.5	81.1	4.7	87.2	0.5
Phenylalanine	90.8	5.4	88.7	1.1	94.8[a]	1.6	83.0[b]	1.9	86.3	3.1	87.7	0.5
Lysine	72.2	8.4	81.4	2.0	73.3	4.9	71.2	3.6	55.6[b]	9.8	81.6[a]	1.0
Histidine	82.4[b]	3.2	93.0[a]	1.1	80.9[b]	2.6	88.4[a]	1.7	73.0[b]	7.1	93.0[a]	0.7
Arginine	90.5	5.9	91.0	0.9	94.9[a]	1.8	85.8[b]	2.1	86.5	3.4	93.3	2.4
Tryptophan	n/a[3]	n/a	n/a	n/a	100.7	1.1	n/a	n/a	97.3	3.8	n/a	n/a

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).

[1]Mean of 4 roosters.

[2]Mean of 6 replicate pens of 8 chicks.

[3] n/a=not analyzed

Table 2.4. Total amino acid concentrations (%) of the six corn distiller's dried grains with or without solubles (DDG(S)), as-fed basis

	DDG(S) Sample number					
Amino acid	1	2	3	4	5	6
Aspartic acid	1.83	1.97	2.11	1.80	4.39	1.67
Threonine	1.03	1.11	1.21	1.02	2.50	0.99
Serine	n/a[1]	n/a	n/a	n/a	3.21	1.17
Glutamic acid	4.70	5.17	5.56	4.70	12.72	3.91
Proline	2.15	2.43	2.61	2.21	5.76	1.94
Alanine	2.00	2.19	2.36	1.98	5.43	1.80
Cysteine	0.51	0.56	0.60	0.51	1.22	0.43
Valine	1.30	1.49	1.57	1.30	3.24	1.24
Methionine	0.54	0.59	0.64	0.54	1.66	0.47
Isoleucine	0.95	1.12	1.20	0.97	2.68	0.94
Leucine	3.14	3.58	3.94	3.22	9.84	2.96
Tyrosine	n/a	n/a	n/a	n/a	3.06	0.95
Phenylalanine	n/a	n/a	n/a	n/a	3.82	1.24
Lysine	0.84	0.93	1.02	0.74	1.81	0.89
Histidine	n/a	n/a	n/a	n/a	1.61	0.69
Arginine	1.30	1.40	1.44	1.24	2.70	1.15
Tryptophan	n/a	n/a	n/a	n/a	0.36	0.18

[1]n/a=not analyzed

Table 2.5. Standardized amino acid digestibility (%) for the six corn distiller's dried grains with solubles (DDGS) samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility assay (SID)

	DDGS Sample number											
	1				2				3			
Amino acid	PFR[1]	SEM	SID[2]	SEM	PFR	SEM	SID	SEM	PFR	SEM	SID	SEM
Aspartic acid	78.9	1.2	67.3	4.0	78.9	1.1	73.3	3.2	77.7	1.3	74.2	2.1
Threonine	79.1	1.0	67.2	3.9	79.1	1.8	72.9	3.5	79.2	2.0	75.1	2.2
Serine	n/a[3]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Glutamic acid	89.6	0.8	84.4	2.0	89.6	0.6	87.6	1.7	88.9	0.9	86.8	1.1
Proline	89.0	0.4	82.5	2.5	89.0[a]	0.9	85.0[b]	1.1	87.5	1.3	86.1	1.0
Alanine	88.1	1.1	70.4	2.4	88.1	0.7	86.5	1.6	87.7	1.0	86.7	1.3
Cysteine	83.1	1.0	87.4	3.3	83.1[b]	1.7	91.7[a]	2.3	82.4[b]	2.3	91.9[a]	1.9
Valine	82.0	0.6	77.9	3.6	82.0	1.4	82.9	2.9	81.3	1.6	82.8	2.3
Methionine	88.9[b]	1.3	96.9[a]	2.4	88.9[b]	0.5	98.4[a]	2.2	89.7[b]	1.4	98.0[a]	1.5
Isoleucine	84.6	1.8	80.6	3.6	84.6	1.1	85.4	2.9	82.0	1.3	85.8	2.2
Leucine	91.7	0.8	86.3	2.4	91.7	0.6	89.5	1.8	91.0	0.8	90.3	1.4
Tyrosine	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Phenylalanine	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	86.7	1.3	n/a	n/a
Lysine	64.3	3.7	68.6	4.9	64.3	1.1	75.1	4.6	64.4	2.6	75.4	3.1
Histidine	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.0
Arginine	86.9	1.1	85.3	3.0	86.9	0.8	88.6	2.6	84.4	1.4	88.4	1.8
Tryptophan	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).

[1]Mean of 4 roosters.

[2]Mean of 6 replicate pens of 8 chicks.

[3] n/a=not analyzed

Table 2.5. contd. Standardized amino acid digestibility (%) for the six corn distiller's dried grains with or without solubles (DDG(S)) samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility assay (SID)

	DDG or DDGS Sample number											
	4				5[1]				6			
Amino acid	PFR[2]	SEM	SID[3]	SEM	PFR	SEM	SID	SEM	PFR	SEM	SID	SEM
Aspartic acid	80.4	1.7	70.1	4.5	94.2[a]	0.8	71.5[b]	1.9	74.6[a]	0.59	67.4[b]	0.6
Threonine	80.7	1.3	68.9	5.1	95.7[a]	0.8	73.0[b]	2.0	76.6[a]	0.52	67.1[b]	0.6
Serine	n/a[4]	n/a	n/a	n/a	96.4[a]	0.6	79.8[b]	2.2	82.5	0.90	78.6	0.9
Glutamic acid	90.8	1.1	86.8	2.0	96.6[a]	0.4	80.9[b]	1.8	85.8[a]	0.59	82.6[b]	1.0
Proline	90.1[a]	1.2	82.5[b]	1.9	96.6[a]	0.5	79.3[b]	1.9	85.1[a]	0.71	80.3[b]	1.0
Alanine	89.8	1.3	84.3	2.7	96.9[a]	0.4	80.6[b]	1.9	86.6[a]	0.55	81.2[b]	0.8
Cysteine	86.1	1.9	89.7	3.2	96.2[a]	0.8	76.8[b]	2.0	79.1[a]	1.86	73.9[b]	0.7
Valine	83.4	1.8	79.7	4.1	95.5[a]	0.6	75.8[b]	2.1	80.3[a]	0.46	74.8[b]	1.1
Methionine	91.0	1.3	98.2	2.6	98.1[a]	0.2	84.9[b]	1.6	87.2[a]	1.03	82.3[b]	1.0
Isoleucine	85.8	2.0	82.5	4.1	96.6[a]	0.4	78.1[b]	2.0	82.3[a]	0.93	76.4[b]	0.9
Leucine	93.1	1.0	88.3	2.5	97.3[a]	0.3	81.0[b]	1.9	91.0[a]	0.43	83.9[b]	1.0
Tyrosine	n/a	n/a	n/a	n/a	97.5[a]	0.3	82.6[b]	1.7	86.5[a]	0.43	81.9[b]	0.8
Phenylalanine	89.7	1.5	n/a	n/a	97.3[a]	0.3	80.9[b]	1.9	87.2[a]	0.67	81.0[b]	0.9
Lysine	63.2	6.0	69.3	6.2	92.9[a]	0.6	73.0[b]	1.7	67.3[a]	1.08	62.5[b]	0.4
Histidine	n/a	n/a	n/a	n/a	94.9[a]	0.4	77.2[b]	1.9	81.1	0.44	78.7	0.8
Arginine	88.7	1.7	86.4	3.1	97.5[a]	0.4	81.3[b]	1.6	86.8[a]	0.74	77.2[b]	0.6
Tryptophan	n/a	n/a	n/a	n/a	96.0[a]	0.8	79.6[b]	1.4	87.4[a]	0.44	79.5[b]	0.6

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).

[1]Previously published Applegate et al. (2009).

[2]Mean of 4 roosters.

[3]Mean of 6 replicate pens of 8 chicks.

[4] n/a=not analyzed

Table 2.6. Total amino acid concentrations (%) and standardized amino acid digestibility (%) for wet distiller's dried grains (WDG) and condensed distiller's solubles (CDS) determined by the precision-fed cecectomized rooster assay.

	DDGS 7-WDG			DDGS 8-CDS		
Amino acid	Total AA	PFR[1]	SEM	Total AA	PFR[1]	SEM
Aspartic acid	2.24	82.8	1.2	1.43	83.0	1.7
Threonine	1.29	84.0	1.7	0.80	82.6	3.3
Serine	1.59	87.6	1.3	0.98	85.7	3.0
Glutamic acid	5.53	90.4	0.9	3.60	90.6	1.3
Proline	2.74	90.2	1.2	1.78	90.3	2.5
Alanine	2.58	91.3	0.9	0.85	90.4	1.4
Cysteine	0.65	87.9	1.9	1.63	84.7	3.7
Valine	1.72	85.9	1.7	0.42	83.2	3.0
Methionine	0.70	92.4	1.2	1.09	90.0	1.2
Isoleucine	1.32	88.8	1.2	0.44	90.0	2.5
Leucine	4.18	94.1	0.7	0.83	94.3	1.4
Tyrosine	1.32	89.3	1.2	2.66	88.4	2.5
Phenylalanine	1.75	90.9	1.1	0.80	92.3	1.9
Lysine	1.17	73.6	3.4	1.12	77.8	4.2
Histidine	0.85	84.4	1.9	0.73	88.2	1.2
Arginine	1.41	89.7	1.4	0.56	89.5	2.9
Tryptophan	0.20	90.1	0.7	0.93	73.3	1.4

[1]Mean of 4 roosters.

Table 2.7. Total amino acid concentrations (%) of two meat and bone meal samples (MBM) and a poultry by-product meal (PBPM)

Amino acid	MBM 1	MBM 2	PBPM
Aspartic acid	3.43	2.65	5.43
Threonine	1.46	1.07	2.58
Serine	1.51	1.27	2.52
Glutamic acid	6.37	4.47	8.37
Proline	3.51	3.38	3.95
Alanine	3.33	3.12	4.59
Cysteine	0.29	0.18	0.70
Valine	2.08	1.48	3.28
Methionine	0.60	0.42	1.12
Isoleucine	1.51	1.04	2.46
Leucine	3.09	2.08	4.96
Tyrosine	1.17	0.73	1.68
Phenylalanine	1.77	1.17	2.72
Lysine	2.21	1.92	4.14
Histidine	0.96	0.57	1.71
Arginine	2.83	2.60	4.26
Tryptophan	0.36	0.26	0.48

Table 2.8. Standardized amino acid digestibility (%) for the two meat and bone meal (MBM) and a poultry by-product meal (PBPM) sample determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility assay (SID)

	MBM 1				MBM 2				PBPM			
Amino acid	PFR[1]	SEM	SID[2]	SEM	PFR	SEM	SID	SEM	PFR	SEM	SID	SEM
Aspartic acid	47.8	6.2	37.8	4.1	51.1	4.7	60.9	2.3	77.2[a]	2.6	40.1[b]	2.4
Threonine	63.2[a]	3.9	48.0[b]	3.0	72.3	3.3	69.7	1.6	86.3[a]	1.5	65.4[b]	1.9
Serine	62.1	4.2	55.9	3.4	69.0	2.8	73.5	1.8	82.9[a]	2.0	71.5[b]	1.8
Glutamic acid	67.8	2.5	61.4	2.8	71.8[b]	1.5	80.9[a]	1.0	85.5[a]	1.4	77.5[b]	1.3
Proline	67.7	3.1	66.0	3.0	66.6[b]	2.1	79.2[a]	1.4	81.7[a]	1.8	73.5[b]	1.2
Alanine	70.1	2.2	63.0	2.7	74.3[b]	1.3	82.6[a]	0.9	86.9[a]	1.2	71.8[b]	1.5
Cysteine	39.7	12.0	27.4	4.2	32.9	10.4	19.0	7.7	69.9	3.7	65.9	1.5
Valine	67.4[a]	3.0	53.3[b]	2.6	75.2	1.9	74.7	1.3	85.6[a]	1.3	66.5[b]	2.0
Methionine	67.9[a]	2.7	45.6[b]	2.9	79.4	1.2	76.6	0.9	89.9[a]	0.9	75.8[b]	1.1
Isoleucine	70.9[a]	2.3	53.9[b]	2.6	80.7	1.3	76.9	1.1	87.4[a]	1.1	68.9[b]	1.9
Leucine	71.1[a]	2.4	53.9[b]	2.3	79.8	1.2	76.4	0.9	88.1[a]	1.1	80.9[b]	1.2
Tyrosine	67.2[a]	2.7	53.3[b]	2.4	74.4	2.7	68.4	1.4	84.8[a]	1.4	77.8[b]	1.4
Phenylalanine	72.9[a]	2.1	56.6[b]	2.2	78.3	1.1	75.2	0.9	87.6[a]	1.1	74.4[b]	1.6
Lysine	49.6	2.2	47.4	3.3	62.5[b]	4.0	76.1[a]	1.6	78.8[a]	2.8	44.6[b]	3.0
Histidine	56.7	2.4	55.6	2.7	62.2[b]	3.1	82.7[a]	1.4	80.9[a]	2.7	69.6[b]	1.9
Arginine	74.5[a]	1.8	64.6[b]	2.5	77.4[b]	1.3	83.6[a]	0.9	88.4[a]	1.2	66.2[b]	2.0
Tryptophan	87.0	0.7	n/a[3]	n/a	87.8	0.8	n/a	n/a	91.7[a]	0.9	73.9[b]	0.6

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).

[1]Mean of 4 roosters.

[2]Mean of 6 replicate pens of 8 chicks.

[3] n/a=not analyzed

Chapter 3

DEVELOPMENT OF A PRECISION-FED ILEAL AMINO ACID DIGESTIBILITY ASSAY UTILIZING 3-WEEK-OLD BROILER CHICKS

ABSTRACT

The objective of this study was to develop a precision-fed ileal digestibility assay, primarily for amino acids, using 3-week old chicks. Day old chicks were fed a standard corn-soybean meal diet until 22 days of age in all experiments. In Experiment 1, feed was removed and excreta were collected at 2, 4, 6, 8, 10, 12, and 14 hours post-feed withdrawal. Results indicated that 8 hours of feed withdrawal was sufficient to empty the ileum of feed residues. In subsequent experiments, cross-bred (New Hampshire x Columbian) or commercial broiler chicks (Cobb or Ross) were fasted overnight and then tube-fed 6, 9, 12, or 15 g of a corn-soybean meal mixture (60:40). Ileal digesta from Meckel's diverticulum to the ileo-cecal junction were then collected at 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0 hours post-feeding. Results indicated that the amount of digesta in the ileum was generally maximized by 4.0 hours post-feeding. In addition, the amount of digesta in the ileum was maximized by feed intakes of 9 g or greater. Apparent and standardized ileal digestibility of amino acids in the corn-soybean meal mixture was determined at 2.5, 3.0, 3.5 and 4.0 hours post feeding. Digestibility values were similar for the 3.0, 3.5, and 4.0 hour collection times, but were generally lower at the 2.5 hour collection time. The results of this study indicate that ileal amino acid digestibility can be determined in 3-week old broiler chicks using a precision-fed assay. For such an assay, it is recommended that the chicks be fasted for at least 8 hours prior to tube-feeding, that the amount of diet precision-fed should be approximately 10 g, and that the ileal contents be collected at approximately 4 hours post-feeding.

INTRODUCTION

The most commonly used methods for determining amino acid digestibility in poultry are the precision-fed cecectomized rooster assay (Parsons, 2002; Ravindran and Bryden, 1999) and the standardized ileal amino acid digestibility assay (SID; Garcia et al., 2007; Parsons, 2002; Payne et al., 1968**)** due to their relative simplicity, precision, and accuracy. The precision-fed cecectomized rooster assay utilizes adult Single Comb White Leghorn roosters that have undergone surgery to have their ceca removed. Removal of the ceca allows for a more accurate estimate of amino acid digestibility due to its ability to eliminate most microbial influences (Ravindran and Bryden, 1999). Excreta are then quantitatively collected over a 48 hour period and analyzed for amino acids. Research has shown that the cecectomized rooster assay yields consistent results, and cecectomized birds were found to have consistently lower amino acid digestibilities in comparison to intact birds (Garcia et al., 2007; Parsons, 2002). However, this procedure requires surgery which may be difficult in some cases and because the surgical procedures must be approved by institutional animal care and use committees. The precision-fed cecectomized rooster assay is also criticized because the birds are being tube-fed which is not normal feeding behavior and the digestibility values may not be accurate for very young animals (Garcia et al., 2007). An alternative to the precision-fed cecectomized rooster assay is a chick bioassay in which ileal digesta are collected (Payne et al., 1968). By estimating the apparent ileal amino acid digestibility and then correcting for basal endogenous amino acid losses, standardized amino acid digestibility coefficients can be determined (Lemme et al., 2004). In this assay, a large number of chicks are usually fed a practical corn-soybean meal diet from 0 to approximately 17 days of age and then are

fed a semi-purified diet containing the test ingredient as the sole source of protein or amino acids from 18 to 21 days of age (Adedokun, et al., 2008). Endogenous amino acid losses are usually determined by either feeding graded levels of casein or a nitrogen-free diet. When compared to the cecectomized rooster assay, the SID requires larger amounts of feedstuff and larger animal numbers and is more expensive and labor intensive, but would better mimic natural feeding behaviors and could be more applicable to the nutrition of younger animals (Adedokun et al., 2008; Garcia et al., 2007; Lemme et al., 2004).

In consideration of the limitations of the cecectomized rooster and SID, a new bioassay utilizing the precision-feeding of three week-old chicks to measure ileal amino acid digestibility is proposed. These animals would be crop-intubated or tube-fed; thus, a smaller amount of sample would be required and there would not be a need to mix the semi-purified diets that are fed for 3-4 days in the SID. Also, there would be no need for surgery as in the cecectomized rooster assay. The objective of this study was to develop a precision-fed ileal amino acid digestibility assay using 3-week-old chicks. This assay would hopefully be complementary to the cecectomized rooster and SID and provide another method that is very rapid, flexible, and convenient to use. The new precision-fed ileal chick assay may also be useful for determining apparent or true metabolizable energy of feed ingredients in chicks if excreta rather than ileal contents are collected. In order to carry out these objectives, a series of experiments were conducted. The first experiment was conducted to determine the length of fasting needed to empty the gastrointestinal tract of feed residues from previously consumed feed. A second experiment was then conducted to determine the optimal amount of feed required to yield

the maximal amount of digesta in the distal small intestine as well as determining the length of time needed post-feeding to maximize the amount of digesta in the distal small intestine. A third experiment determined the basal endogenous amino acid losses so that both apparent and standardized amino acid digestibility coefficients from a corn: soybean mixture could be calculated. A final experiment was conducted using both cross-bred and commercial broiler strains to determine apparent and standardized amino acid digestibility coefficients at several collection periods post-feeding.

MATERIALS AND METHODS

All studies involving animals were approved by the University of Illinois Institutional Animal Care and Use Committee.

Experiment 1-Determination of the Length of Pre-Experimental Fasting Period Needed to Empty the Ileum of Feed Residues from Previously Consumed Feed

Six 22 day-old broiler chicks (Ross 308) were wing-banded and placed into individual cages measuring 11 x 8.5 x 8.25 inches and deprived of feed for 14 hours. Animals were allowed free access to water during this time. Trays were placed under the cages and excreta were collected every two hours for the entire 14-hour fasting period. After collection, the excreta were frozen, freeze-dried and weighed for each individual chick.

Experiment 2-Determination of the Optimum Feeding Amount and Ileal Digesta Collection Time

Sixty day-old broiler chicks (Ross 308) were fed a nutritionally-complete chick starter diet until 21 days. On Day 22, the chicks were fasted for 14 hours overnight. This fasting period was determined from results from the previous experiment. Four chicks

were then randomly allocated among each of 15 different treatments that were based on the varying combinations of amount of feed that the animals were precision-fed (6,9,12, or 15 g) and the time that the ileal digesta were collected post-feeding (3.0, 3.5, 4.0, 4.5, 5.0, 6.0, or 7.0 hours). The 15 g treatment group had collection times extended past 5 hours to determine if a longer collection time would increase the amount of ileal digesta. All chicks were precision-fed a corn-soybean meal (60:40) mixture with chromic oxide added at 0.3% as an indigestible marker. The precision-feeding of the chicks was modified from methodology developed by Sibbald (1976) and Parsons (1985). The intubation equipment consisted of a plastic funnel (2.25 inches in diameter) fused to a brass tube measuring 9.25 inches in length with a diameter of 0.25 inches. The tube was placed into the esophagus and the feed mixture was placed into the tube and pushed into the crop with a steel rod, measuring 9.25 inches in length with a diameter of 0.23 inches. The stainless steel rod was extended approximately 4.0 inches at the top to form a handle and to allow the feed mixture to be pushed into the crop. A metal washer was fused to the rod at 9.25 inches from the end of the rod to prevent the end of the rod from being pushed beyond the bottom of the brass feeding tube into the crop. Each treatment group of chicks was then placed into a battery and the chicks had free access to water. The chicks were then euthanized via CO_2 inhalation at the different times post-feeding. Ileal digesta were collected from Meckel's diverticulum to the ileo-cecal junction. Ileal digesta were freeze-dried and weighed for each individual chick.

Experiment 3-Determination of the Ileal Endogenous Amino Acid Losses for Precision-fed Chicks

Twenty-four day-old broiler chicks (Ross 308) were placed on a nutritionally complete starter diet until day 21. On day 21, they were fasted overnight and then randomly allocated into 4 groups of 6 chicks. Each chick was then precision-fed 10 g of a nitrogen-free diet (NFD) using the methods from the previous experiment. The composition of the NFD is presented in Table 3.1. At four hours post-feeding, the chicks were euthanized via CO_2 inhalation and the ileal digesta were collected and pooled for the 6 chicks within each of the 4 replicate groups. The ileal digesta were then freeze-dried, ground and analyzed for amino acids and chromium [method 990.08 (AOAC International, 2000)] at the Experiment Station Chemical Laboratories, University of Missouri-Columbia. The ileal endogenous amino acid flow (IEAA) was calculated as milligrams of amino acid per kilogram of feed dry matter intake (DMI) using the following formula (Moughan et al., 1992):

Endogenous amino acid flow, mg/kg of DMI = [(amino acid in ileal digesta, mg/kg) x [(diet chromium, mg/kg) / ileal chromium, mg/kg]]

Ileal digesta were analyzed for amino acids [method 982.30 E (a, b, c; AOAC International, 2000)] at the Experiment Station Chemical Laboratories, University of Missouri-Columbia.

Experiment 4-Determination of Apparent and Standardized Ileal Amino Acid Digestibility of a Corn:Soybean mixture

A final experiment was conducted to determine the ileal amino acid digestibility of a corn-soybean mixture (60:40) in 21-day-old chicks at different collection times post-

feeding. Forty day-old chicks were fed a nutritionally complete starter diet until day 21. Twenty of the chicks were cross-bred (New Hampshire x Columbian) male chicks and twenty chicks were a male commercial broiler strain (Cobb). On day 21, they were fasted overnight and then randomly allocated into treatment groups based on varying collection times post-feeding. Ileal digesta were collected at 2.5, 3.0, 3.5, and 4.0 hours post-feeding. All chicks were precision-fed 10 g of the corn-soybean mixture and ileal digesta were collected at the various times post-feeding. There were 2 replicate groups (one crossbred and one Cobb) of 5 chicks. Assigned to each collection period, each treatment group of chicks was then placed into a battery and the chicks had free access to water. The chicks were then euthanized via CO_2 inhalation at the different times post-feeding. Ileal digesta were collected from Meckel's diverticulum to the ileo-cecal junction. Ileal digesta were pooled for the 5 chicks in each group, freeze-dried, and analyzed for amino acids [method 982.30 E (a, b, c; AOAC International, 2000)] and chromium [method 990.08 (AOAC International, 2000)] at the Experiment Station Chemical Laboratories, University of Missouri-Columbia. Apparent ileal amino acid digestibility coefficients were calculated using the following formula (Moughan et al., 1992). The apparent ileal amino acid digestibility coefficients were standardized using two different methods; the IEAA flows from 21 d old broiler chicks ad libitum fed a nitrogen-free diet (Adedokun et al., 2007) or the IEAA flows from the previous experiment.

APPARENT ILEAL AMINO ACID DIGESTIBILITY, % =

[1 − (chromium in diet/chromium in ileal digesta) × (amino acid in digesta/amino acid in diet)] × 100

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =

Apparent digestibility + [(IEAA flow, g/kg of DMI)/(amino acid content of the diet, g/kg of DM)] × 100.

STATISTICAL ANALYSIS

Data from all experiments were analyzed using the ANOVA procedure of SAS (SAS Institute, 1990) for completely randomized designs. Statistical significance of differences among individual treatments was assessed using the least significant difference test (Carmer and Walker, 1985).

RESULTS AND DISCUSSION

The results from the fasting study (Experiment 1) are shown in Figure 3.1. The amount of excreta voided decreased steadily until 8 hours post-feeding. At 8 hours post-fasting, the amount of excreta had reached a plateau and there was no significant decrease in excreta after 8 hours. It was concluded that the removal of feed for 8 hours is a sufficient amount of time for 3 week-old broiler chicks to empty their gastrointestinal tracts of feed residues. Based on the results of this preliminary study, at least 8 hours of fasting was used prior to precision-feeding chicks for all subsequent studies.

The effect of the precision-feeding amount and ileal collection time (Experiment 2) are presented in Table 3.2. For chicks fed 6, 9, or 12 g, the amount of digesta in the ileum was found to be either significantly ($P < 0.05$) or numerically increased at 4 hours when compared to 3 hours. Extending the collection time beyond 4 hours had no significant effect on the amount of digesta recovered at the distal small intestine for any amount that was precision-fed. Dry ileal digesta weight was generally higher for chicks fed 9, 12, or 15 g when compared to chicks in the 6 g treatment groups. Feeding chicks

12 or 15 g yielded variable results, which was evident by the larger SEMs for these treatment groups. The large variation for the chicks fed 12 or 15 g may have been a result of several of the latter chicks having very little digesta in the ileum, even at 7 hours post-feeding. It is hypothesized that precision-feeding amounts of 12 and 15 g may have been too high and caused the feed to compact inside the crop. Since the feed may have become compacted, it may not have been able to move through the gastrointestinal tract during the allotted collection period which is undesirable for this type of assay. Based on the results of this experiment, precision-feeding chicks approximately 9 g yielded the maximal and the most consistent amounts of ileal digesta after a 4 hour collection period. The earliest collection time for the feed to yield maximal amounts of digesta in the ileum was 4 hours post-feeding. Maximizing the amount of ileal digesta collected is important to provide an adequate amount of sample to conduct amino acid and digesta marker analyses in order to determine digestibility.

The ileal endogenous amino acid flow (IEAA) is presented in Table 3.3 along with values determined in a previous study by Adedokun et al. (2007) for 21-day-old broiler chicks that were fed a NFD ad libitum. The IEAA flow for the precision-fed chicks was generally higher in the current study for chicks fasted for 8 hours and precision-fed 10 g of NFD than for ad libitum fed chicks in the earlier study. The reason for this difference is unknown but there may be several explanations. The ad libitum fed chicks were allowed free access to the NFD for 5 days in the Adedokun et al. (2007) study whereas chicks in the current study were tube-fed only 10 g of NFD after an overnight fasting period. Moter and Stein (2004) reported that as feed intake increased, the IEAA flow was found to decrease when expressed relative to the dry matter intake

(DMI) for growing pigs. Since the precision-fed chicks were tube-fed a small amount of NFD (low intake), this may be one explanation for the high IEAA of the tube-fed chicks when compared with ad libitum fed chicks. Another explanation for the increased IEAA flow in precision-fed chicks may be due to the period of fasting prior to feeding. Thompson and Applegate (2006) reported that as periods of feed withdrawal increased for broilers, the morphology of the small intestine was affected by decreasing villi width and crypt depth and decreasing mucin secretion. Thompson and Applegate (2006) concluded that these changes reduced the integrity of the intestine. These alterations of the small intestine enterocyte morphology may also partially explain the differences in IEAA flow for precision-fed versus ad libitum fed chicks.

The apparent amino acid digestibilities of a 10 g of 60:40 corn-soybean meal mixture (Experiment 4) were generally found to be numerically or significantly decreased at 2.5 hours post-feeding when compared to the later collection times of 3.0, 3.5, or 4.0 hours (Table 3.4). Using basal endogenous IEAA flow values determined in Experiment 3 and previously by Adedokun et al. (2007; Table 3.3), standardized amino acid digestibilities were calculated (Table 3.5). As observed earlier for apparent digestibility values, the standardized amino acid digestibilities were numerically or significantly ($P<0.05$) decreased at 2.5 hours post-feeding when compared with the 3.0, 3.5, or 4.0 hours post-feeding for both standardization methods. These results indicate that collecting ileal contents at 4 hours post-feeding is a good collection time because it yields consistent digestibility values and a greater amount of ileal digesta than 3 hours post-feeding (Table 3.2). When comparing the two standardization methods, digestibility values for cysteine, methionine, lysine, and tyrosine were significantly higher ($P<0.05$)

when standardized using IEAA flow from chicks precision-fed a NFD and all other amino acids were numerically higher than when standardized using IEAA flows from ad libitum fed chicks. The precision-fed NFD method also yielded some digestibility values in excess of 100%. These results suggest that using the IEAA flows from chicks fed a NFD ad libitum may be more suitable for standardizing amino acid digestibility values in the new precision-fed method developed herein, particularly when feeding ingredients that are low in protein.

In conclusion, a new precision-fed ileal amino acid digestibility assay was developed. Ileal amino acid digestibility can be determined using 3-week-old chicks by fasting the animals for at least 8 hours, precision-feeding approximately 10 g of feed, and collecting ileal digesta at 4 hours after feeding. This new assay is relatively inexpensive, rapid, requires only a small amount of feed ingredient and does not require mixing semi-purified diets. Thus, the new assay provides a flexible, convenient, non-surgical, and complementary alternative to the cecectomized rooster and SID for determine amino acid digestibility in feed ingredients for poultry.

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Table 3.1. Composition of the nitrogen-free diet[1]

Ingredient	(%)
Cornstarch	28.25
Dextrose	56.49
Solkafloc[2]	5.00
Soybean oil	5.00
Vitamin premix[3]	0.20
Mineral premix[4]	0.15
Choline chloride	0.30
NaCl	0.30
Limestone	1.90
Dicalcium phosphate	2.11
Chromic Oxide	0.30

[1] Calculated to contain 0% CP, 3462 kcal/kg TME_n, 0.39% nonphytate P and 1.19% Ca.

[2] Purified cellulose, International Fiber Corp., North Tonawanda, NY.

[3] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-∝-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[4] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \bullet H_2O$; iron, 75 from $FeSO_4 \bullet H_2O$; zinc, 75 from ZnO; copper, 5 from $CuSO_4 \bullet 5H_2O$; iodine, 0.75 from ethylene diamine dihydroiodide; selenium, 09.1 from Na_2SeO_3.

Table 3.2. Effect of post-feeding collection time and precision-feeding amount on dry ileal digesta weights in Experiment 2

Collection time (hr)	Amount fed (g)	Ileal digesta(g)	Pooled SEM[1]
3.0	6.0	0.33	0.125
4.0	6.0	0.42	
5.0	6.0	0.44	
3.0	9.0	0.33[b]	0.071
4.0	9.0	0.70[a]	
5.0	9.0	0.63[a]	
3.0	12.0	0.17	0.191
4.0	12.0	0.63	
5.0	12.0	0.60	
3.0	15.0	0.75	0.189
4.0	15.0	0.67	
4.5	15.0	0.82	
5.0	15.0	0.41	
6.0	15.0	0.36	
7.0	15.0	0.70	

[a-b] Means within a column and feeding amount are significantly different ($P<0.05$).

[1] Values are the means of four chicks. Pooled SEM values are for statistical analyses within amount fed.

Table 3.3. Ileal endogenous amino acid flow (mg/kg dry matter intake) for 21-day-old chicks precision-fed a nitrogen-free diet compared to previously published values for ad libitum-fed chicks in Experiment 3[1]

Amino acid	Precision-fed amino acid flow	SEM	Previously published values[2]	SEM[2]
Aspartic acid	731	66.8	340	54.6
Threonine	629	45.2	274	41.1
Serine	479	35.8	260	53.2
Glutamic acid	888	94.4	420	123.7
Proline	456	33.4	240	36.9
Glycine	369	36.3	205	27.0
Alanine	303	23.6	177	28.6
Cysteine	375	91.5	136	10.5
Valine	400	101.5	214	35.6
Methionine	220	63.7	50	13.2
Ileucine	463	79.9	162	35.1
Leucine	424	45.3	251	42.4
Tyrosine	287	34.7	124	17.2
Phenylalanine	162	56.9	154	21.6
Lysine	358	54.1	181	39.9
Histidine	167	18.7	73	12.0
Arginine	340	40.1	168	28.0
Tryptophan	85	24.6	n/a[3]	n/a

[1]Values are means of four groups of six chicks each.
[2]For 21-d old broiler chicks ad libitum fed a nitrogen-free diet (Adedokun et al., 2007).
[3]n/a=not analyzed

Table 3.4. Apparent ileal amino acid digestibility values (%) for precision-fed 21-day-old chicks at several different collection times after precision-feeding a corn-soybean meal (60:40) mixture in Experiment 4[1]

	Apparent ileal amino acid digestibility				
Amino acid	2.5 hr	3.0 hr	3.5 hr	4.0 hr	Pooled SEM
Aspartic Acid	87.2	91.8	90.1	92.1	1.4
Threonine	76.6	86.3	83.8	88.0	3.4
Serine	84.8	90.7	88.8	91.2	1.8
Glutamic Acid	91.6	95.0	93.7	95.0	0.9
Proline	85.3	91.4	89.6	91.8	2.0
Glycine	83.4	89.9	87.6	90.2	1.8
Alanine	87.4	92.6	90.6	92.5	1.4
Cysteine	76.8	86.1	84.5	86.6	2.7
Valine	85.2	91.2	89.2	92.2	1.9
Methionine	89.8[b]	94.6[a]	92.5[ab]	94.2[a]	0.9
Isoleucine	86.8	92.2	90.3	93.1	1.7
Leucine	87.9	92.6	90.7	92.6	1.4
Tyrosine	85.7[b]	91.6[ab]	89.9[ab]	92.2[a]	1.6
Phenylalanine	85.9[b]	91.4[ab]	89.8[ab]	92.2[a]	1.5
Lysine	88.8	93.6	91.4	93.5	1.4
Histidine	91.6	94.8	93.4	95.2	0.9
Arginine	92.2	95.3	94.0	95.7	1.0
Tryptophan	90.2	94.4	92.9	94.1	1.2

[a,b] Means within a row with no common superscripts are significantly different ($P<0.05$).

[1]Values are means of two groups of five chicks.

Table 3.5.Standardized ileal amino acid digestibility values (%) for precision-fed 21-day-old chicks at several different collection times after precision-feeding a corn-soybean meal (60:40) mixture calculated using two different ileal endogenous amino acid flows[1]

	Standardized Ileal Amino Acid Digestibility (Precision-fed Nitrogen-Free Diet)					Standardized Ileal Amino Acid Digestibility (Ad libitum fed Nitrogen-Free Diet)[1,2]					
Amino Acid	2.5 hr	3.0 hr	3.5 hr	4.0 hr	Pooled SEM	2.5 hr	3.0 hr	3.5 hr	4.0 hr	Pooled SEM	Pooled SEM[3]
Aspartic Acid	89.2^{b}	95.9^{a}	94.2^{a}	96.1^{a}	1.1	89.0	93.7	92.0	93.9	1.4	0.8
Threonine	81.2^{b}	95.5^{a}	93.0^{a}	97.2^{a}	2.3	80.6	90.3	87.8	92.0	3.4	1.9
Serine	87.8^{b}	96.7^{a}	94.8^{a}	97.3^{a}	1.4	88.1	94.0	92.1	94.5	1.8	1.1
Glutamic Acid	93.0^{b}	97.8^{a}	96.5^{ab}	97.8^{a}	0.9	92.9	96.3	95.0	96.3	0.9	0.6
Proline	87.4^{b}	95.6^{a}	93.8^{a}	96.0^{a}	1.5	87.5	93.6	91.8	94.0	1.8	1.0
Glycine	85.9^{b}	95.0^{a}	92.7^{a}	95.2^{a}	1.5	86.2	92.7	90.4	93.0	2.0	1.1
Alanine	89.1^{b}	95.9^{a}	93.9^{ab}	95.8^{a}	1.4	89.4	94.5	92.5	94.4	1.4	0.9
Cysteine[4]	82.8^{b}	98.2^{a}	96.6^{a}	98.6^{a}	2.1	81.2	90.5	88.9	90.9	2.7	1.6
Valine	87.5^{b}	95.9^{a}	93.9^{a}	96.9^{a}	1.6	87.7	93.7	91.7	94.7	1.9	1.1
Methionine[4]	93.2^{b}	101.5^{a}	99.4^{a}	101.1^{a}	1.5	91.4^{b}	96.2^{a}	94.1^{ab}	95.8^{a}	1.3	0.9
Isoleucine	90.0^{b}	98.5^{a}	96.7^{a}	99.4^{a}	1.6	89.0	94.4	92.5	95.3	1.7	1.1
Leucine	89.2^{b}	95.3^{a}	93.3^{ab}	95.2^{a}	1.3	89.4	94.2	92.3	94.2	1.4	0.8
Tyrosine[4]	88.1^{b}	96.5^{a}	94.8^{a}	97.1^{a}	1.4	87.8^{b}	93.7^{ab}	92.0^{ab}	94.3^{a}	1.6	1.0
Phenylalanine	86.8^{b}	93.2^{a}	91.6^{a}	94.0^{a}	1.3	87.7^{b}	93.1^{ab}	91.5^{ab}	93.9^{a}	1.5	0.9
Lysine[4]	92.5^{b}	101.1^{a}	98.8^{ab}	101.0^{a}	1.8	92.6	97.4	95.2	97.3	1.4	1.1
Histidine	92.5^{b}	96.5^{a}	95.2^{ab}	96.9^{a}	0.9	92.4	95.6	94.2	95.9	0.9	0.6
Arginine	93.6^{b}	98.1^{a}	96.8^{ab}	98.5^{a}	1.0	93.6	96.7	95.4	97.1	1.0	0.6
Tryptophan	91.9^{b}	98.0^{a}	96.4^{a}	97.6^{a}	0.9	n/a[5]	n/a	n/a	n/a	n/a	n/a

[a,b] Means within a row and standardization method with no common superscripts are significantly different (P<0.05).

[1]Values are means of two groups of four chicks.

[2] Endogenous amino acid flow from Adedokun et al. (2007) used for calculation of standardized ileal amino acid digestibility.

[3]Overall SEM for comparison of feeding methods (Precision-fed vs. Ad-libitum fed). Values for each time are means of two groups of four chicks.

[4] Significant difference among amino acid digestibility coefficients between the two standardization methods.

[5]n/a= not analyzed.

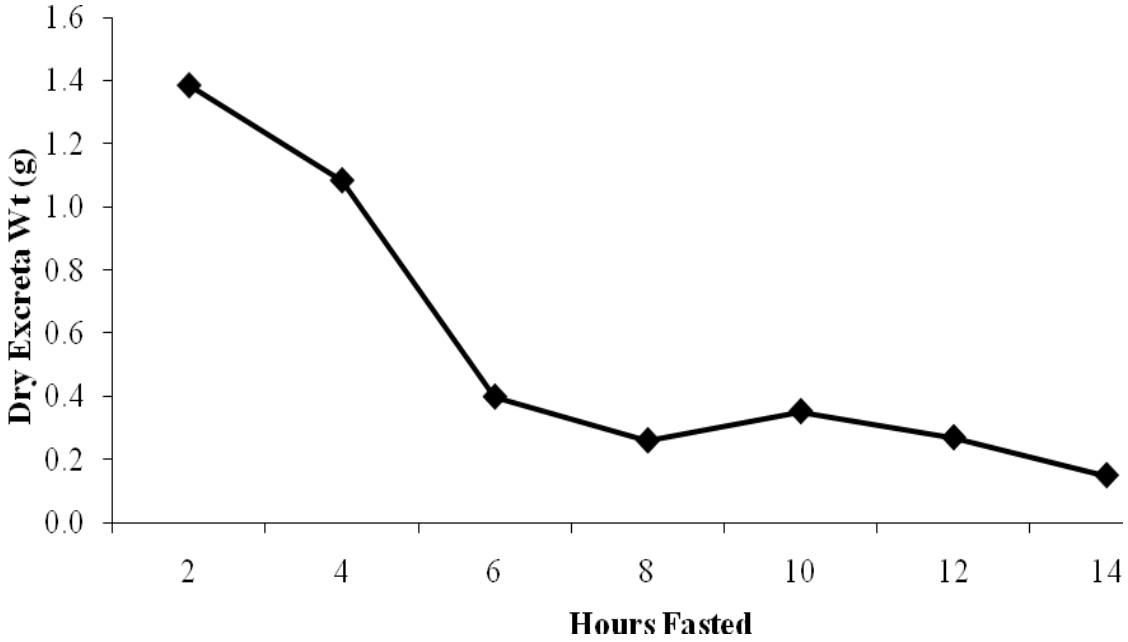

Figure 3.1. Mean excreta weights for broiler chicks fasted for 14 hours. Values are means of six chicks each. (Pooled SEM =0.05)

Chapter 4

COMPARISON OF AMINO ACID DIGESTIBILITY COEFFICIENTS FOR CORN, CORN GLUTEN MEAL, AND CORN DISTILLER'S DRIED GRAINS WITH SOLUBLES (DDGS) AMONG THREE DIFFERENT BIOASSAYS

ABSTRACT

The objective of this study was to determine standardized amino acid digestibility of corn and several corn by-products (corn gluten meal (CGM) and three DDGS samples) using the precision-fed cecectomized rooster assay (PFR), the standardized ileal chick assay (SID), and a newly developed precision-fed ileal chick assay (PFC). For the PFR, cecectomized roosters were precision-fed approximately 30 g of feed sample and excreta were collected 48 hours post-feeding. For the SID, 16 day-old chicks were ad libitum fed a semi-purified diet containing the feed samples as the sole source of protein from 17-21 d, with ileal digesta collected at 21 d. For the PFC, 22 day-old chicks were precision-fed 10 g of feed and ileal digesta were collected at 4 hours post-feeding. For corn, the PFC yielded significantly higher digestibilities than the SID and PFR for several amino acids. For the CGM, the PFR yielded significantly higher values than the SID and PFC for the majority of the amino acids. When three DDGS samples were evaluated, the PFR produced higher digestibilities than the PFC for all three DDGS samples for most of the amino acids. When comparing the PFR and the SID, the PFR yielded higher values than the SID for one DDGS, whereas there was no significant difference between the two methods for the other two DDGS samples. Among the amino acids in DDGS, Lys had the widest range in digestibility among the methods. The results of this study indicate there were differences among standardized amino acid digestibility values for the PFR, SID, and PFC in some instances but that the differences among methods were not consistent.

INTRODUCTION

Cereal grains, such as corn, are primarily used in poultry diets as a source of carbohydrates and energy. Due to its abundance in the United States, corn is widely used in poultry diets. Since corn is such a good source of starch, it has also been used extensively for ethanol production. In the last decade, ethanol production from corn has increased exponentially. Ethanol can be produced by two different methods, the wet-milling or dry grind process, with the latter being the focus of the increased ethanol production in recent years (Gibson and Karges, 2006; Singh et al., 2005). The large increase in dry-grind ethanol production is also creating a proportional increase in corn distiller's dried grains with solubles (DDGS). Corn gluten meal (CGM) is another important co-product derived from corn that is produced through the wet-milling process. The CGM is an ideal ingredient because of its high protein content (60%; NRC, 1994), high energy content, and high level of xanthophylls, which is attractive to layer and broiler producers because CGM can be used to enhance skin and yolk pigmentation (Peter et al., 2000). Methionine or sulfur amino acids are the first limiting amino acids in a corn-soybean meal diet for poultry (Fernandez et al., 1994), and CGM has been found to be a highly available source of methionine (Sasse and Baker, 1973). However, it has an imbalanced amino acid profile, being severely deficient in lysine, tryptophan, and arginine (Peter et al., 2000).

In order to evaluate AA digestibility of feedstuffs, the precision-fed cecectomized rooster assay (PFR) is a widely accepted method of determining AA digestibility of feedstuffs in poultry (Parsons et al., 1982; Ravindran and Bryden, 1999). The largest constraint of this assay is the need to surgically modify adult roosters in order to remove

the ceca (Parsons et al., 1982). In addition, there is some concern that this rooster assay may not accurately estimate AA digestibility for younger animals (Garcia et al., 2007; Ravindran and Bryden, 1999). In response to some of these concerns, the standardized ileal chick assay (SID) was developed using 3-week-old broilers (Lemme et al., 2004). By ad libitum feeding a semi-purified diet with the feedstuff providing all the crude protein in the SID, the chicks display a more normal feeding behavior than the PFR. This SID was developed on the premise that digesta collected at the distal portion of the ileum would accurately estimate digestibility in broiler chicks (Payne et al., 1968). This type of assay is more expensive, time-consuming, and labor intensive than the PFR (Garcia et al., 2007). In order to address concerns involving both assays, a new precision-fed ileal chick assay (PFC) was developed. In this type of assay, 3-week-old broilers are fasted, precision-fed a feedstuff and ileal digesta are collected 4 hours post-feeding. The PFC should provide a precision-feeding assay with chicks that is rapid, cost-effective and complementary to the SID while hopefully yielding digestibility values that are similar to the SID and PFR. Therefore, the objective of this study was to compare amino acid digestibility determined using the PFR, SID, and PFC. To carry out this objective corn, CGM, and three different DDGS samples were obtained and evaluated in all three assays.

MATERIALS AND METHODS

Feed Sample Analysis

A corn, CGM, and three DDGS samples were obtained. The three DDGS samples were obtained from different plants. All feedstuffs were evaluated for N and amino acids (AOAC International, 2000: method 99n/a3, 982.30 E (a, b, c) at the Experiment Station Chemical Laboratories, University of Missouri-Columbia.

Standardized Ileal Amino Acid Digestibility Chick Assay (SID)

All animal care, handling, and euthanasia procedures for this and subsequent experiments were approved by the University of Illinois at Urbana-Champaign Animal Care and Use Committee. Male Ross 308 broiler chicks were obtained at 1 d of age from a commercial hatchery and fed a nutritionally complete starter diet until d 16. After overnight fasting, the birds were weighed individually and randomized to 5 dietary treatments, with 5 birds per pen, 4 replicate pens per experimental diet. The birds were fed the 5 experimental diets for a five day period. On d 21, birds were killed by CO_2 asphyxiation and ileal digesta were collected.

SID Diet Formulation

The experimental diets were formulated to contain approximately 20% CP (with the exception of the corn diet, which was approximately 7% CP), with each of the feedstuffs supplying the entire CP in the diets. The experimental diets were also formulated to meet nutritional requirements in energy, vitamins, and minerals for 3-week-old broilers (NRC, 1994). All feedstuffs were analyzed for CP prior to diet formulation. Chromic oxide was added to all diets as an indigestible marker at 0.30% of the diet, with all diets being fed in mash form. Composition of the experimental diets are presented in Table 4.1.

Precision-fed Cecectomized Rooster Assay (PFR)

A precision-fed rooster assay utilizing cecectomized Single Comb White Leghorn roosters were conducted (Parsons, 1985). After 24 hours of feed withdrawal, four cecectomized roosters were tube fed approximately 30 grams of each of 5 feed samples.

Excreta were then quantitatively collected for 48 hours. Endogenous corrections for amino acids were made using excreta from roosters that had been fasted for 48 hours.

Precision-fed Ileal Amino Acid Chick Assay (PFC)

Male Ross 308 broiler chicks were obtained at 1 d of age and fed a standard starter diet until day 21. Feed was removed from the chicks for an overnight period of at least 8 hours to ensure the lower gastrointestinal tract was emptied of feed residues. Chicks were individually weighed and randomized into 4 groups of 4 chicks. Each chick was then precision-fed 10 grams of each of the 5 feed samples. Each replicate group was then placed into a battery cage and the chicks were allowed free access to water. Four hours after feeding, the chicks were euthanized via CO_2 asphyxiation and ileal digesta were collected.

Sampling, Ileal Digesta, and Excreta Processing

For the SID and the PFC, the contents of the ileum were considered to be the part of the small intestine from the Meckel's diverticulum to the approximately 1 cm proximal to the ileo-cecal junction. The ileal digesta from birds within pens or groups were pooled, frozen, and stored at -20ºC until they were processed. For the rooster assay, the excreta were also frozen and stored at -20ºC until processing. All ileal and excreta samples were freeze-dried, ground by using a mortar and pestle and then sent to the University of Missouri Experiment Station and Chemical Laboratories for amino acid and chromium analysis (only SID and PFC digesta were analyzed for chromium) (AOAC International, 2000: method 99n/a8, ICP method).

Calculations

Amino acid digestibility for the SID and PFC were calculated using the following formulas by Moughan et al. (1992). The apparent ileal amino acid digestibility coefficients obtained from the SID were standardized by using ileal endogenous amino acid (IEAA) flow values from 21 d-old broiler chicks fed a nitrogen-free diet (Adedokun et al., 2007). The apparent ileal amino acid digestibility coefficients obtained from the PFC were standardized using IEAA values from chicks precision-fed a nitrogen-free diet (Kim, Chapter 3). For the SID, the diet was the semi-purified diet with the feed ingredient as the sole source of protein. The diets for the PFC and PFR were the feed ingredient itself.

APPARENT ILEAL AMINO ACID DIGESTIBILITY =

[1 − (chromium in diet/chromium in ileal digesta) × (amino acid in digesta/amino acid in diet)]

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =

Apparent digestibility + [(IEAA flow, g/kg of DMI)/(amino acid content of the diet, g/kg of DM)] × 100.

For the rooster assay, standardized amino acid digestibility values were calculated with the following formula. The amino acids were standardized using an endogenous correction based on amino acids excreted by fasted roosters.

STANDARDIZED AMINO ACID DIGESTIBILITY, %=

[(Amino acid in feed ingredient (mg) − Amino acid excreta (mg) + endogenous amino acid (mg))/ amino acid in feed ingredient (mg)] × 100.

STATISTICAL ANALYSIS

All data from both assays were analyzed by using PROC GLM (SAS Institute, 1990) as a completely randomized design. Differences among treatment means were determined by using the PDIFF option in the least-square means (LSMEAMNS) procedure of GLM. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Total amino acid concentrations for the corn, CGM, and DDGS samples are presented in Table 4.2 and standardized amino acid digestibility coefficients for the five samples are presented in Table 4.3. When the corn sample was fed in the three assays, there was a greater variability (larger SEM) in the SID for amino acid digestibility values when compared to the PFR and SID. When comparing the PFC to the other two methods, it generally yielded numerically higher amino acid digestibility coefficients with the difference being significant ($P<0.05$) for some amino acids. The increased amino acid digestibility may be due to a high endogenous correction being used to standardize the amino acid coefficients which resulted in an overestimation of digestibility of several amino acids (Kim, Chapter 3). For example, standardized Met digestibility was calculated to be 100.8% in comparison while the PFR and the SID yielded lower digestibilities (93.2 and 89.1%, respectively). Due to corn being low in crude protein and amino acid levels (resulting in low intake), any error in the endogenous amino acid correction will have a large effect on amino acid digestibility values. While the PFC yielded higher digestibilities, there were no differences between the PFR and SID for any of the amino acids.

Standardized amino acid digestibility of CGM was greater for the PFR and SID than the PFC for most of the amino acids. Lys was an exception where there were no differences among assays. The PFR also yielded significantly greater digestibility values for several amino acids when compared to the SID.

Total amino acid concentrations of the three DDGS samples are presented in Table 4.2. There was variation among the amino acids for the three samples. For example, total Lys content was found to range from 0.67% (DDGS 2) to 1.01% (DDGS 1). The difference in Lys between these two DDGS samples was greater than that for the other amino acids and this large difference was not expected since there was only a modest difference in crude protein. When calculated as a percentage of CP, the Lys/CP ratio for DDGS 1 and 2 were 3.7% and 2.5%, respectively, suggesting increased heat damage or overheating of DDGS 2 compared with DDGS 1 (Martinez Amezcua and Parsons, 2007; Stein et al., 2009). When comparing amino acid digestibility values (Table 4.4), the PFR consistently yielded higher digestibility values for DDGS 1 when compared to the PFC. The SID also yielded higher values than the PFC for DDGS 1 for most amino acids. The PFR and SID values did not differ except for Met. For DDGS 2, the PFR again yielded significantly higher digestibilities than the PFC for all the amino acids expect Lys. Values for the PFR were also higher than those for the SID for several amino acids. Differences between the SID and PFC were not consistent. The SID yielded a very low digestibility value for Lys (37%) which was lower (P<0.05) than the other two methods. When standardized amino acid digestibilities were determined for DDGS3, values for the PFR and SID were significantly higher than the PFC for several amino

acids, but there were no differences among methods for most of the practically important indispensable amino acids.

Lysine has always been an amino acid of particular interest in DDGS samples due its high variability. In this current study, there was a wide variation of in total Lys and digestibility among the samples and the methods. As mentioned earlier, total Lys content was found to range from 0.67% (DDGS 2) to 1.01% (DDGS 1) (Table 4.2). The NRC (1994) reports a total Lys content of 0.75% in DDGS, which falls within the range of our analyzed values. Similar results have been reported in earlier studies. Batal and Dale (2006) reported total Lys content in eight DDGS samples to range from 0.39% to 0.86%, with an average Lys digestibility of 69.6%. Fastinger et al. (2006) reported total Lys content of five DDGS samples to range from 0.48% to 0.76%, with an average Lys digestibility of 76.6%. More recently, Pahm et al. (2009) reported total Lys content of seven DDGS samples to range between 0.65% to 0.94%, with an average of 0.77% and Lys digestibility averaging 61.4%. DDGS 1 in the current study had a Lys digestibility that ranged from 58% (PFC) to 70% (PFR). DDGS 2 had the lowest Lys digestibility at 37% (SID), but yielded higher values for the other assays (58% for both the PFC and the PFR). DDGS 3 had the lowest range in Lys digestibility, ranging from 64% (PFR) to 66% (SID and PFCs). All three DDGS samples were commercially available samples and were obtained from different plants. All three samples also varied in color. Of the three samples evaluated in this study, DDGS 2 had the darkest color. DDGS 2 also had the lowest Lys digestibility for all three methods evaluated, particularly for the SID. DDGS 2 also had the lowest Lys/crude protein ratio. These results suggest that the DDGS 2 was more heat damaged than DDGS 1 and 3. It has been proposed that the color

of the DDGS may be indicative of lower Lys content and digestibility (Ergul et al., 2003; Fastinger et al., 2006). The darker color may indicate increased levels of Maillard reaction, which is the reaction of a reducing sugar to a free amino group, usually the epsilon amino group of Lys, during heat treatment. During the dry-grind processing scheme, the wet distiller's grains are mixed with the solubles fraction, which contains a high concentration of reducing sugars and the mixture is then dried at high temperatures to decrease the moisture content of the wet grains and solubles (Kwiatkowski et al., 2006). However, this part of the ethanol process is not highly regulated and can produce DDGS of varying color due to differences in drying temperature and length (Belyea et al., 2004). While the drying processes and solubles contents for our three DDGS samples are unknown, they may account for the differences in Lys content and digestibility observed in this study.

Several differences in standardized amino acid digestibility values were observed among the three methods and the reasons for the differences are unknown. The differences among methods were not consistent, although the PFR generally yielded digestibility values that were higher than the PFC. Digestibility values determined with the PFR and SID were generally not significantly different. Ravindran and Bryden (1999) also compared the SID and PFR. For a corn sample, the amino acid digestibility determined by the PFR method and SID were similar, with significant differences only for a few amino acids (Ravindran and Bryden, 1999). In a subsequent study, Garcia et al. (2007) compared amino acid digestibility of several feedstuffs using the PFR and the SID, also including a corn sample. In the corn sample evaluated, most of the digestibilities of the essential amino acids were not significantly different between the PFR and SID,

which is in agreement with this current study. The CGM and DDGS ingredients were not evaluated in the Ravindran and Bryden (1999) and Garcia et al. (2007) studies.

In conclusion, all of the methods evaluated herein (PFR, SID, and the new PFC) seem to be acceptable methods for determining amino acid digestibility in poultry. Differences were sometimes observed among assays, but these differences were not consistent. The PFR and SID were generally in good agreement and when differences were observed between these assays, they were generally not large. The new PFC yielded lower digestibility values than the PFR and SID (particularly when compared to the PFR) in several instances and the reason for these results may warrant further investigation.

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Table 4.1. Composition of the experimental diets used in the SID (as-fed basis)[1]

	Diet[2]				
Ingredient (%)	Corn	Corn gluten meal	DDGS1[1]	DDGS 2	DDGS 3
Cornstarch	--	17.99	4.22	3.94	4.70
Dextrose	--	35.74	8.19	7.62	9.16
Feed Ingredient	85.18	31.34	73.56	74.40	72.07
Solkafloc[3]	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00
Vitamin premix[4]	0.20	0.20	0.20	0.20	0.20
Mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.30	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30	0.30
Limestone	1.23	1.20	1.63	1.65	1.63
Dicalcium phosphate	2.34	2.47	1.15	1.14	1.19
Chromic oxide	0.30	0.30	0.30	0.30	0.30

[1] SID=standardized ileal chick assay; DDGS=distiller's dried grains with solubles

[1] All diets were calculated to contain 20% CP, a minimum 0.50% Available P and a minimum 1.00% Ca, except the corn diet

[3] Purified cellulose, International Fiber Corp., North Tonawanda, NY.

[4] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-∝-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[5] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \bullet H_2O$; iron, 75 from $FeSO_4 \bullet H_2O$; zinc, 75 from ZnO; copper, 5 from $CuSO_4 \bullet 5H_2O$; iodine, 0.75 from ethylene diamine dihydroiodide; selenium, 09.1 from Na_2SeO_3.

Table 4.2.Total amino acid concentration (%) of corn, corn gluten meal (CGM), and three corn distiller's dried grains with solubles (DDGS), as fed basis.

Amino acid	Corn	CGM	DDGS 1	DDGS 2	DDGS 3
Alanine	0.53	5.39	1.96	1.89	1.82
Arginine	0.35	2.19	1.23	1.03	1.27
Aspartic acid	0.49	3.86	1.81	1.56	1.60
Cysteine	0.17	1.20	0.59	0.53	0.54
Glutamic acid	1.28	12.64	4.41	4.15	3.31
Histidine	0.20	1.32	0.77	0.64	0.71
Isoleucine	0.26	2.58	1.03	0.84	0.99
Leucine	0.88	10.32	3.20	2.84	3.07
Lysine	0.24	1.15	1.01	0.67	0.85
Methionine	0.16	1.67	0.54	0.43	0.51
Phenylalanine	0.36	3.98	1.22	1.04	1.28
Proline	0.59	5.46	2.04	1.92	1.80
Serine	0.32	2.64	1.19	1.11	1.10
Threonine	0.26	2.04	1.02	0.90	0.98
Tyrosine	0.23	3.23	1.00	0.86	0.95
Valine	0.34	2.95	1.42	1.21	1.35
Crude Protein	7.56	63.81	27.19	26.88	27.75

Table 4.3. Comparison of standardized amino acid digestibilities (%) for corn and corn gluten meal determined by three different methods

	Corn						Corn gluten meal					
Amino acid	SID[1]	SEM	PFC[2]	SEM	PFR[3]	SEM	SID	SEM	PFC	SEM	PFR	SEM
Alanine	89.8	2.3	91.2	0.4	91.7	1.2	94.0^{a}	0.9	85.6^{b}	0.7	95.8^{a}	0.2
Arginine	87.0	3.7	93.1	0.6	91.5	2.1	90.6^{ab}	2.1	87.0^{b}	1.0	93.1^{a}	0.7
Aspartic acid	82.3	4.9	90.1	1.5	88.2	2.0	85.8^{b}	1.3	82.1^{c}	1.1	91.2^{a}	0.4
Cysteine	88.0	2.6	93.2	1.3	94.0	3.1	85.0^{b}	0.7	74.0^{b}	1.7	87.9^{a}	0.9
Glutamic acid	91.0	2.0	93.6	1.0	92.5	1.1	93.5^{b}	0.9	85.0^{c}	0.7	95.8^{a}	0.2
Histidine	85.3^{a}	2.5	89.9^{a}	1.0	76.4^{b}	1.9	88.6^{a}	1.3	81.3^{b}	1.0	89.5^{a}	0.7
Isoleucine	85.6^{b}	4.3	95.2^{a}	0.6	88.2^{b}	1.9	90.7^{a}	1.6	83.4^{b}	0.9	93.4^{a}	0.5
Leucine	91.2	2.0	91.7	0.7	94.7	1.3	94.7^{a}	0.9	85.4^{b}	0.7	96.8^{a}	0.2
Lysine	74.4^{b}	7.6	94.6^{a}	0.4	74.5^{b}	2.5	81.9	4.9	85.2	1.4	84.2	0.8
Methionine	89.1^{b}	3.8	100.8^{a}	0.5	93.2^{ab}	1.8	93.3^{b}	1.4	87.3^{c}	0.6	96.5^{a}	0.2
Phenylalanine	89.4	2.9	87.3	0.6	92.7	1.9	93.3^{a}	1.1	85.6^{b}	0.6	95.5^{a}	0.3
Proline	90.6	1.3	89.2	1.0	93.5	1.3	92.6^{b}	0.6	82.0^{c}	0.7	94.9^{a}	0.4
Serine	84.6	3.5	92.1	1.9	92.6	2.7	90.3^{a}	0.9	85.3^{b}	1.2	92.4^{a}	0.7
Threonine	76.5	4.6	79.8	2.8	90.8	2.0	83.9^{b}	1.1	81.7^{b}	1.4	90.8^{a}	0.8
Tyrosine	87.0^{ab}	2.6	92.2^{a}	0.6	86.3^{b}	2.2	92.8^{b}	1.0	87.8^{c}	0.6	95.2^{a}	0.4
Valine	79.6	3.7	87.4	0.9	87.4	2.2	86.8^{b}	0.6	81.7^{c}	1.0	92.6^{a}	0.5

^{a,b} Means within a row within sample without common superscripts are significantly different ($P<0.05$).

[1]SID=Standardized ileal chick assay; mean of 4 replicate pens of 5 chicks.

[2]PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 chicks.

[3]PFR=Precision-fed cecectomized rooster assay; mean of 4 roosters.

Table 4.4. Comparison of standardized amino acid digestibilities (%) for three different corn distiller's dried grains with solubles (DDGS) samples determined by three different methods

	DDGS 1						DDGS 2					
Amino acid	SID[1]	SEM	PFC[2]	SEM	PFR	SEM	SID	SEM	PFC	SEM	PFR	SEM
Alanine	80.7[ab]	1.3	73.7[b]	3.6	83.4[a]	0.3	78.5[a]	0.7	73.3[b]	1.1	80.9[a]	0.5
Arginine	77.6[ab]	1.5	73.4[b]	3.3	83.2[a]	0.7	69.3[b]	1.5	70.6[b]	1.0	78.8[a]	0.3
Aspartic acid	67.9[a]	1.6	58.0[b]	4.7	73.8[a]	0.9	58.4[b]	1.2	54.5[b]	0.7	66.6[a]	0.8
Cysteine	72.7[a]	1.7	53.3[b]	6.0	77.6[a]	1.6	62.5[b]	1.2	49.0[c]	1.3	68.9[a]	1.0
Glutamic acid	79.1[ab]	1.5	72.6[b]	4.0	85.3[a]	0.5	74.9[b]	0.8	69.4[c]	1.1	80.7[a]	0.1
Histidine	74.4[a]	1.5	64.2[b]	4.9	78.3[a]	0.5	62.7[b]	1.2	60.2[b]	1.0	67.6[a]	0.6
Isoleucine	72.7[ab]	1.4	66.4[b]	4.6	78.4[a]	0.5	58.9[c]	1.8	65.2[b]	1.0	73.6[a]	0.4
Leucine	82.1[ab]	1.4	74.7[b]	3.8	88.2[a]	0.4	79.2[b]	0.8	74.0[c]	1.2	86.8[a]	0.2
Lysine	61.7[a]	2.4	58.3[b]	4.3	69.5[a]	0.4	37.0[b]	3.6	57.6[a]	1.1	57.5[a]	1.2
Methionine	78.4[b]	1.3	76.8[b]	3.3	86.2[a]	0.2	69.7[c]	1.6	74.5[b]	1.5	83.3[a]	0.6
Phenylalanine	80.9[a]	1.0	70.7[b]	4.0	82.1[a]	0.7	75.4[a]	1.2	68.8[b]	1.1	79.4[a]	0.1
Proline	80.2[a]	1.5	68.3[b]	4.7	84.1[a]	0.9	75.8[a]	0.8	68.6[b]	1.2	79.1[a]	0.4
Serine	77.1[a]	1.7	68.0[b]	4.1	77.4[a]	0.8	74.6[a]	0.8	67.2[b]	0.7	75.5[a]	2.1
Threonine	66.4[ab]	2.0	55.7[b]	5.4	70.5[a]	2.0	61.3[b]	1.1	56.8[b]	1.1	67.5[a]	1.4
Tyrosine	81.6[ab]	1.2	74.7[b]	3.6	82.4[a]	0.5	77.4[a]	1.0	72.2[b]	1.0	78.7[a]	0.4
Valine	71.7[ab]	1.6	63.5[b]	4.7	78.5[a]	0.6	60.6[b]	1.4	62.2[b]	0.9	73.5[a]	1.1

[a,b] Means within a row within sample without common superscripts are significantly different ($P<0.05$).

[1]SID=Standardized ileal chick assay; mean of 4 replicate pens of 5 chicks.

[2]PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 chicks.

[3]PFR=Precision-fed cecectomized rooster assay; mean of 4 roosters.

Table 4.4 (cont). Comparison of standardized amino acid digestibilities (%) for three different corn distiller's dried grains with solubles (DDGS) samples determined by three different methods

	DDGS 3					
Amino acid	SID	SEM	PFC	SEM	PFR	SEM
Alanine	84.6[a]	0.5	78.7[b]	1.8	82.9[a]	1.0
Arginine	81.5	1.6	80.2	1.7	82.7	1.3
Aspartic acid	72.1[a]	1.2	63.9[b]	2.6	71.0[a]	1.6
Cysteine	80.9[a]	0.6	65.2[b]	3.9	75.3[a]	2.7
Glutamic acid	84.7[a]	0.6	75.6[b]	2.5	81.3[a]	1.2
Histidine	80.9[a]	0.5	74.6[b]	2.3	78.1[ab]	0.8
Isoleucine	78.8	1.2	74.6	2.1	79.1	1.4
Leucine	86.8[a]	0.5	81.9[b]	1.7	88.3[a]	0.9
Lysine	65.9	3.1	65.6	2.2	63.5	1.5
Methionine	84.5	1.3	81.8	1.7	85.2	1.1
Phenylalanine	84.8[a]	1.1	77.8[b]	1.9	84.6[a]	1.1
Proline	85.2[a]	0.1	75.1[b]	2.7	82.8[a]	1.2
Serine	81.5	0.7	76.8	2.2	76.4	2.3
Threonine	73.0	1.2	69.1	2.7	72.6	1.2
Tyrosine	84.9	0.9	81.3	1.8	82.8	1.3
Valine	77.6	0.9	73.4	2.4	78.2	1.4

[a,b] Means within a row within sample without common superscripts are significantly different ($P<0.05$).

[1]SID=Standardized ileal chick assay; mean of 4 replicate pens of 5 chicks.

[2]PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 chicks.

[3]PFR=Precision-fed cecectomized rooster assay; mean of 4 roosters.

Chapter 5

COMPARISON OF AMINO ACID DIGESTIBILITY COEFFICIENTS FOR SOYBEAN MEAL, CANOLA MEAL, FISH MEAL, AND MEAT AND BONE MEAL AMONG THREE DIFFERENT BIOASSAYS

ABSTRACT

The objective of this study was to determine amino acid digestibility of various feedstuffs (soybean meal (SBM), canola meal, fish meal, and meat and bone meal (MBM)) using the precision-fed cecectomized rooster assay (PFR), the standardized ileal assay (SID), and a newly developed precision-fed chick assay (PFC). For the PFR, cecectomized roosters were precision-fed approximately 30 g of feed sample and excreta were collected 48 hours post-feeding. For the SID, 16 day-old chicks were fed a semi-purified diet containing the feed samples as the only source of protein from 17-21 d, with ileal digesta collected at 21 d. For the PFC, 22 day-old chicks were precision-fed 10 g of feed sample mixed with chromic oxide and ileal digesta were collected at 4 hours post-feeding. Digestibility coefficients were standardized using a nitrogen-free diet (NFD) for the SID and PFCs and using fasted roosters for the PFR. There were generally no consistent differences in standardized amino acid digestibility values among assays and values were in general agreement among assays, particularly for SBM and MBM. Differences did occur among methods for amino acid digestibility in fish meal; however, these differences were not consistent among methods or amino acids. The results of the study indicated that all three bioassays are acceptable for determining the amino acid digestibility of SBM, canola meal, MBM, and fish meal for poultry.

INTRODUCTION

Two common protein sources used in poultry diets are soybean meal and canola meal. Soybean meal is a good protein source due to its large supply as well as its high protein content (48.5%; NRC, 1994) and consistent amino acid profile. However, soybean meal also contains antinutritional factors such as trypsin inhibitors and lectins. These factors can be inactivated by heating the soybeans during processing (Liener, 1994). These heat processing conditions must be carefully monitored or reduced amino acid digestibility may occur due to inadequate or excessive heating conditions. Canola meal is another plant protein source that is commonly used in the poultry industry. It has a relatively high crude protein content (38%; NRC, 1994) but a somewhat low metabolizable energy value (2,070 kcal/kg; NRC, 1994). Canola meal has lower amino acid digestibility and protein content compared with soybean meal (Zuprizal et al., 1992). This lower amino acid digestibility may be caused by the desolventization and toasting stage of the prepress solvent extraction of canola (Newkirk and Classen, 1999; Newkirk et al., 2000). It is hypothesized that the desolventizing and toasting stage during processing can remove some of the antinutritional factors present in canola, but may decrease lysine content and digestibility in canola meal (Newkirk and Classen, 1999). Animal-based protein sources are also widely used in poultry production, with the U.S. poultry industry utilizing approximately 37% of total animal by-products produced by the rendering industry (Pearl, 2002). These types of protein supplements are desirable due to their high protein and often high phosphorus content (NRC, 1994). Most animal-based protein sources, such as meat and bone meal, are animal products recovered after livestock slaughter and are further processed by the rendering industry for use as animal feeds. The rendering process converts the raw materials into protein rich granular feedstuffs by the process of drying and fat extraction (Pearl, 2002). Proteins in hair, hide, and

bone can be hard to digest because they are high in keratin and other collagenous protein, which can be partially denatured during the heating process. However, prolonged drying time or overheating can reduce the protein quality. Animal-based protein sources can also be highly variable among batches, which may influence final amino acid content and digestibility. Variability of these feedstuffs will be affected by origin of the raw material as well as differences in processing (Ravindran and Bryden, 1999). Fish meal is another attractive animal-based protein source and is attractive for feed formulations because of its high protein content (60%; NRC, 1994) and generally high amino acid digestibility. However, fish meal can be undesirable because it can impart off-flavors to meat and egg products and can be subject to the same types of nutrient variability as meat and bone meal since both types are usually processed under similar conditions.

To evaluate amino acid digestibility of feedstuffs, the precision-fed cecectomized rooster assay (PFR) is a widely accepted method of determining amino acid digestibility of feedstuffs in poultry (Parsons et al., 1982; Ravindran and Bryden, 1999). The largest constraint of this assay is the need to surgically modify adult roosters in order to remove the ceca (Parsons et al., 1982). In addition, there is some concern that this rooster assay may not accurately estimate amino acid digestibility for younger animals (Garcia et al., 2007Ravindran and Bryden, 1999). In response to some of these concerns, the standardized ileal chick assay (SID) was developed using 3-week-old broilers (Lemme et al., 2004). By ad libitum feeding a semi-purified diet with the feedstuff providing all the crude protein in the SID, the chicks display a more normal feeding behavior. This assay was developed on the premise that digesta collected at the distal portion of the ileum would accurately estimate amino acid digestibility in broiler chicks (Payne et al., 1968). This type of assay is more expensive, time-consuming, and labor intensive in than the PFR (Garcia et

al., 2007). To address concerns involving both assays, a new precision-fed ileal chick assay (PFC) was developed. In this type of assay, 3-week-old broilers are fasted, precision-fed a feedstuff and ileal digesta are collected 4 hours post-feeding. The PFC should provide an assay with chicks that is rapid, cost effective and complementary to the SID while hopefully yielding amino acid digestibility values that are similar to the SID and PFR.

The objective of this study was to determine and compare amino acid digestibility among the PFR, SID, and new PFC. To accomplish this objective, samples of plant and animal based protein sources, namely soybean meal, canola meal, meat and bone meal (MBM), and fish meal were obtained and evaluated. These samples represent a broad spectrum of protein sources used in the poultry industry.

MATERIALS AND METHODS

Feed Sample Analysis

A soybean meal, canola meal, fish meal, and MBM sample were obtained. All feedstuffs were analyzed for N and amino acids (AOAC International, 2000: method 99n/a3, 982.30 E (a, b, c) at the Experiment Station Chemical Laboratories, University of Missouri-Columbia.

Standardized Ileal Digestibility Chick Assay (SID)

All animal care, handling, and euthanasia procedures for this bioassay and all subsequent bioassays were approved by the University of Illinois at Urbana-Champaign Animal Care and Use Committee. This assay was conducted using the procedures described by Adedokun et al. (2008). Male Ross 308 broiler chicks were obtained at 1 d of age from a commercial hatchery and fed a nutritionally complete starter diet until d 16 before they were placed on the experimental diets. After an overnight period of fasting, birds were weighed individually and randomized to 4 dietary treatments, with 5 birds per pen, 4 replicate pens per experimental diet.

The birds were fed the 5 experimental diets for a five day period. On d 21, birds were killed by CO_2 asphyxiation and ileal digesta were collected.

Diet Formulation

The semi-purified experimental diets (Adedokun et al., 2008) fed in the SID were formulated to contain approximately 20% CP, with each of the feedstuffs supplying the only source of CP in the diets. The experimental diets were also formulated to meet the nutritional requirements in energy, vitamins, and minerals for 3-week-old broiler (NRC, 1994). All the feedstuffs were analyzed for CP before diet formulation. Chromic oxide was added to all the diets as an indigestible marker at 0.30% of the diet, with all diets being fed in mash form. Compositions of the experimental diets are presented in Table 5.1.

Precision-fed Cecectomized Rooster Assay (PFR)

A precision-fed rooster assay utilizing cecectomized Single Comb White Leghorn roosters was conducted (Parsons, 1985). After 24 hours of feed withdrawal, four cecectomized roosters were tube fed approximately 30 grams of each of 4 feed samples. Excreta were then quantitatively collected for 48 hours in both assays. Endogenous corrections for amino acids were made using excreta from roosters that had been fasted for 48 hours.

Precision-fed Ileal Amino Acid Chick Assay (PFC)

Male Ross 308 broiler chicks were obtained at 1 d of age and fed a standard starter diet until day 21. Feed was removed from the chicks for an overnight period of at least 8 hours to ensure the lower gastrointestinal tract was emptied of feed residues. Four groups of four chicks were then precision-fed 10 grams of each of the 4 feed samples. Each replicate group was then placed into a battery cage and the chicks were allowed free access to water. Four hours after feeding, the chicks were euthanized via CO_2 asphyxiation and ileal digesta were collected.

Sampling, Ileal Digesta, and Excreta Processing

For the SID chick assay and the PFC, the contents of the ileum were considered to be the part of the small intestine from the Meckel's diverticulum to the approximately 1 cm proximal to the ileo-cecal junction. The ileal digesta from birds within pens or groups were pooled, frozen, and stored at -20ºC until they were processed. For the rooster assay, the excreta were also frozen and stored at -20ºC until processing. All ileal and excreta samples were freeze-dried, ground by using a mortar and pestle and then sent to the University of Missouri Experiment Station and Chemical Laboratories for amino acid and chromium analysis (only SID and PFC digesta were analyzed for chromium) (AOAC International, 2000: method 99n/a8, ICP method).

Calculations

Amino acid digestibility for the SID and PFC were calculated using the following formulas by Moughan et al. (1992). The apparent ileal amino acid digestibility coefficients obtained from the SID were standardized by using ileal endogenous amino acid (IEAA) flow values from 21 d-old broiler chicks fed a nitrogen-free diet (Adedokun et al., 2007). The apparent ileal amino acid digestibility coefficients obtained from the PFC were standardized using IEAA values from chicks precision-fed a nitrogen-free diet (Kim, Chapter 3).

APPARENT ILEAL AMINO ACID DIGESTIBILITY, % =

[1 − (chromium in diet/chromium in ileal digesta) × (amino acid in digesta/amino acid in diet)]

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =

apparent digestibility, % + [(IEAA flow, g/kg of DMI)/(amino acid content of the raw material, g/kg of DM)] × 100.

For the rooster assay, standardized amino acid digestibility values were calculated with the following formula. The amino acids were standardized using an endogenous correction based on amino acid excreta from fasted roosters.

STANDARDIZED AMINO ACID DIGESTIBILITY, %=

[(Amino acid in feed (mg) − Amino acid excreta (mg) + endogenous Amino acid (mg))/ Amino acid in feed (mg)] × 100.

STATISTICAL ANALYSIS

All data from all three assays were analyzed by using PROC GLM (SAS Institute, 1990) as a completely randomized design. Differences among treatment means were determined using the PDIFF option in the least-square means (LSMEANS) procedure of GLM. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The total amino acid concentrations in the soybean meal, canola meal, fish meal, and meat and bone meal (Table 5.2) were in general agreement with published table values (NRC, 1994). The standardized amino acid digestibility coefficients of soybean meal and canola meal determined by three different methods are presented in Table 5.3. For soybean meal, there was very little variation among the methods. The only difference among methods was that Arg and His digestibility in soybean meal was increased in the PFC when compared with the PFR and Asp was significantly increased in the PFR when compared with the SID. For canola meal, the SID yielded more variable results in comparison to the other two methods which were evident by the increased SEM for this assay. The PFR generally yielded greater amino acid digestibilities than the PFC with the SID being intermediate. For Ala, Lys, and His, there were no significant differences among the three methods.

The standardized amino acid digestibilities of fish meal and MBM determined by three different methods are presented in Table 5.4. There were significant differences in digestibility coefficients for the majority of the amino acids in fish meal when they were determined by the three different methods, but these differences were not consistent and did not indicate any type of pattern. For example, Thr digestibility was significantly increased in the PFR and PFC when compared with the SID. However, Met digestibility was greatest in the PFR (92%) assay and least in the PFC (83%) with the SID yielding an intermediate digestibility (88%). Conversely, for Lys digestibility, the PFC value was greater than the value for PFR but not the SID. For MBM, the majority of the amino acid digestibility values (with the exception of Cys, His, Met, Pro and Ser) were not different among the methods and the latter differences were not consistent. For example, Met digestibility was significantly higher in the PFC and PFR when compared to the SID. In contrast, Pro and His digestibility was increased in the SID when compared to the PFR, indicating there was no clear pattern in predicting differences among methods.

Ravindran and Bryden (1999) summarized a few studies that compared ileal and excreta amino acid digestibility of broilers fed a wide range of feed ingredients. For SBM, there were generally no significant differences; however, some differences between excreta and ileal digestibility were observed for a few individual amino acids. Interestingly, the animal protein sources (meat meal, fish meal, and feather meal) were found to have consistently greater digestibility for the excreta than the ileal method. Differences between excreta and ileal digestibility were most evident for MBM, particularly with the essential amino acids Thr and Val. The significant differences between the excreta and ileal digestibility may have largely been due to hindgut microflora modification of the amino acid excretion; thus, ileal digestibility may be a more accurate measure of amino acid digestibility in animal protein sources. Garcia et al. (2007)

reported that the PFR yielded significantly higher digestibilities than the SID for most amino acids in SBM and fish meal. In a similar study, Adedokun et al. (2009) also compared ileal amino acid digestibility of broilers and excreta amino acid digestibility of cecectomized roosters of several feedstuffs. The PFR consistently yielded significantly higher amino acid digestibilities for SBM, canola meal, and MBM samples when compared to the SID chick assay (Adedokun et al., 2009). Overall the results of the above studies suggest that the PFR yields greater amino acid digestibility than the SID. These results of these previous studies are not in agreement with the results of this current study where there were no consistent differences between the PFR and the two ileal assays (SID and PFC) for SBM, fish meal, and canola meal ($P<0.05$).

In conclusion, the PFR, SID and the newly developed PFC all seem to be acceptable methods for determining amino acid digestibility of feedstuffs for poultry. Significant differences were sometimes observed in amino acid digestibility among the methods, but these differences were not consistent among methods or amino acids.

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Table 5.1 Composition of the experimental diets used in the SID (as-fed basis)[1]

	Diet[2]			
Ingredient (%)	SBM	Canola meal	Fish meal	MBM
Cornstarch	15.17	11.82	19.13	16.26
Dextrose	29.48	25.39	38.00	32.26
Feed ingredient	40.90	51.03	31.62	36.83
Solkafloc[3]	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00
Vitamin premix[4]	0.20	0.20	0.20	0.20
Mineral premix[5]	0.15	0.15	0.15	0.15
Choline chloride	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
Limestone	1.10	0.63	--	--
Dicalcium phosphate	2.10	1.88	--	--
Chromic oxide	0.30	0.30	0.30	--
Sodium bicarbonate	--	--	--	2.00
KCl	--	--	--	1.20
MgO	--	--	--	0.20

[1] SID=standardized ileal chick assay; SBM=soybean meal; MBM=meat and bone meal

[2] All diets were calculated to contain 20% CP, a minimum of 0.50% Available P and a minimum 1.00% Ca

[3] Purified cellulose, International Fiber Corp., North Tonawanda, NY.

[4] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-∝-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[5] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \bullet H_2O$; iron, 75 from $FeSO_4 \bullet H_2O$; zinc, 75 from ZnO; copper, 5 from $CuSO_4 \bullet 5H_2O$; iodine, 0.75 from ethylene diamine dihydroiodide; selenium, 09.1 from Na_2SeO_3.

Table 5.2. Total amino acid concentrations, crude protein, and dry matter (%) of soybean meal (SBM), canola meal, fish meal, and meat and bone meal (MBM), as-fed basis

Amino acid	SBM	Canola meal	Fish meal	MBM
Alanine	2.04	1.70	4.03	3.83
Arginine	3.53	2.55	3.82	3.67
Aspartic acid	5.52	3.37	5.79	4.03
Cysteine	0.77	0.87	0.60	0.47
Glutamic acid	8.56	6.66	8.30	6.47
Glycine	2.00	1.81	4.80	7.11
Histidine	1.26	1.03	1.45	1.03
Isoleucine	2.26	1.64	2.66	1.52
Leucine	3.78	2.87	4.61	3.27
Lysine	3.13	2.31	4.98	2.93
Methionine	0.68	0.72	1.80	0.75
Phenylalanine	2.40	1.74	2.43	1.75
Proline	2.21	2.04	2.70	3.90
Serine	2.06	1.53	2.10	1.78
Threonine	1.82	1.59	2.49	1.61
Tryptophan	0.70	0.50	1.95	0.36
Tyrosine	1.78	1.16	3.14	1.28
Valine	2.40	2.00	4.03	2.29
Crude Protein	48.94	39.19	63.25	54.31

Table 5.3. Comparison of standardized amino acid digestibilities (%) of soybean and canola meals determined by three different methods

	Soybean meal						Canola meal					
Amino acid	SID[1]	SEM	PFC[2]	SEM	PFR[3]	SEM	SID	SEM	PFC	SEM	PFR	SEM
Alanine	87.3	0.9	87.1	1.1	84.4	1.8	78.1	2.7	73.5	1.1	77.1	0.4
Arginine	91.7[ab]	0.5	91.1[a]	1.0	88.8[b]	1.4	84.6[ab]	2.0	81.1[b]	1.0	88.8[a]	1.0
Aspartic acid	86.3[b]	0.4	87.3[ab]	1.1	89.8[a]	1.2	76.8[b]	2.1	73.0[b]	0.7	82.0[a]	0.7
Cysteine	83.0	0.6	88.4	1.2	85.9	2.4	77.0[ab]	2.1	71.6[b]	1.3	81.7[a]	0.9
Glutamic acid	90.3	0.4	91.7	1.1	91.8	1.1	85.3[ab]	1.7	82.7[b]	1.1	86.8[a]	0.6
Histidine	89.4[ab]	0.7	91.6[a]	1.1	87.5[b]	1.3	82.0	2.0	78.1	1.0	80.7	1.3
Isoleucine	87.0	0.6	94.1	1.0	89.1	1.4	76.8[a]	2.5	71.6[b]	1.0	81.5[a]	0.4
Leucine	87.5	0.6	89.6	1.2	88.8	1.5	78.6[ab]	2.5	72.8[b]	1.2	81.5[a]	0.5
Lysine	89.1	1.0	88.5	1.0	88.0	1.8	76.9	3.0	75.1	1.1	78.9	2.0
Methionine	90.4	1.4	87.7	1.4	90.9	1.3	81.9[ab]	2.7	80.9[b]	1.5	86.4[a]	0.2
Phenylalanine	88.5	0.5	91.4	1.2	90.4	1.3	80.2[a]	2.3	73.4[b]	1.1	84.8[a]	0.6
Proline	88.4	0.5	89.8	1.1	87.0	1.5	78.8[a]	1.7	72.4[b]	1.2	80.9[a]	1.0
Serine	88.9	0.6	88.6	1.3	88.2	1.8	77.9	2.3	76.1	0.7	79.0[a]	1.1
Threonine	85.1	0.8	86.6	1.3	85.1	2.0	73.4[ab]	2.6	69.8[b]	1.1	79.3[a]	0.6
Tyrosine	88.9	0.6	87.8	1.0	90.2	1.2	77.5[ab]	2.5	73.8[b]	1.0	81.4[a]	0.8
Valine	85.8	0.8	86.2	1.2	86.9	1.7	75.7[ab]	2.6	70.9[b]	0.9	80.2[a]	0.5

[a,b] Means within a row within sample without common superscripts are significantly different ($P<0.05$).

[1]SID=Standardized ileal chick assay; mean of 4 replicate pens of 5 chicks.

[2]PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 chicks.

[3]PFR=Precision-fed cecectomized rooster assay; mean of 4 roosters.

Table 5.4. Comparison of standardized amino acid digestibilities (%) of fish meal and meat and bone meal determined by three different methods

	Fish meal						Meat and bone meal					
Amino acid	SID[1]	SEM	PFC[2]	SEM	PFR[3]	SEM	SID	SEM	PFC	SEM	PFR	SEM
Alanine	87.4[b]	0.4	97.1[a]	0.1	87.7[b]	0.3	84.7	1.5	81.8	1.4	81.2	1.0
Arginine	88.1[b]	0.4	90.3[a]	0.5	89.7[a]	1.3	84.8	2.2	84.9	1.2	83.6	0.9
Aspartic acid	79.1[b]	0.7	81.4[b]	1.1	85.1[a]	0.6	71.3	3.0	69.1	2.4	68.3	1.3
Cysteine	76.1[a]	1.4	37.7[b]	3.4	76.8[a]	1.3	63.9[b]	6.1	75.5[a]	2.6	64.8[ab]	1.7
Glutamic acid	87.0[b]	0.5	88.9[a]	0.6	88.9[a]	1.7	79.6	2.5	80.3	1.6	79.0	0.5
Histidine	84.4[b]	0.9	87.9[a]	0.7	81.6[c]	3.1	73.5[a]	2.8	75.8[a]	1.6	66.3[b]	0.7
Isoleucine	85.2[b]	0.6	82.0[c]	0.9	90.9[a]	4.2	73.6	4.6	80.5	1.7	81.8	1.1
Leucine	87.0[c]	0.6	95.7[a]	0.3	91.0[b]	0.9	77.7	3.6	80.0	1.5	82.9	1.0
Lysine	87.2[ab]	0.5	89.6[a]	0.6	86.8[b]	0.6	76.1	4.0	80.0	1.5	73.1	2.3
Methionine	87.7[b]	0.4	82.9[c]	0.9	92.4[a]	0.2	74.0[b]	4.8	80.8[ab]	1.8	84.9[a]	0.8
Phenylalanine	84.7[c]	0.7	98.4[a]	0.2	89.2[b]	0.4	78.4	3.6	78.2	1.3	80.6	0.8
Proline	85.1[a]	0.6	84.4[ab]	1.0	82.3[b]	0.2	83.5[a]	1.0	77.5[b]	1.9	76.5[b]	0.8
Serine	83.5	1.2	84.8	0.7	85.0	1.3	80.7[a]	2.2	76.6[ab]	1.6	73.2[b]	0.8
Threonine	84.1[b]	0.7	87.2[a]	0.8	88.4[a]	1.6	75.4	4.3	77.0	1.8	77.1	0.7
Tyrosine	84.6[b]	0.8	84.0[b]	1.0	90.0[a]	2.0	73.7	4.4	79.2	1.6	77.8	1.2
Valine	84.4[c]	0.8	94.5[a]	0.3	89.0[b]	0.3	76.3	3.5	79.6	1.5	80.5	0.8

[a-c] Means within a row within sample without common superscripts are significantly different ($P<0.05$).

[1]SID=Standardized ileal chick assay; mean of 4 replicate pens of 5 chicks.

[2]PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 chicks.

[3]PFR=Precision-fed cecectomized rooster assay; mean of 4 roosters.

Chapter 6

SUMMARY AND CONCLUSIONS

Amino acid digestibility of feedstuffs is important in poultry nutrition. Formulating diets on digestible amino acids can be beneficial to producers. One of the most widely used and accepted methods of determining amino acid digestibility in feedstuffs is the precision-fed cecectomized rooster assay (PFR). In this assay, adult roosters undergo a surgical procedure to have their ceca removed. After recovery, they are fasted, precision-fed a feed ingredient (usually 30 g), and excreta are collected over a period of 48 hours and analyzed for amino acids. Digestibilities are usually standardized using excreta amino acids from fasted animals. This assay has been criticized for its abnormal feeding method and that surgically-modified animals are needed. Consequently, the standardized ileal chick assay (SID) was proposed. For this assay, broiler chicks (usually 3-week-old) are ad libitum fed a semi-purified diet containing the test ingredient as the sole source of protein for several days. At the end of the feeding period, the chicks are euthanized and ileal digesta are collected for amino acid analysis. Digestibilities are usually standardized by ad libitum feeding a nitrogen-free diet. In comparison to the PFR, this assay does not require surgery, but requires a larger amount of feedstuff as well as larger animal numbers and is more labor intensive and expensive due to time of rearing birds within the facility. Few studies have compared standardized amino acid digestibilities between the PFR and SID methods. For the first study (Chapter 2), 15 samples of several feedstuffs were obtained and amino acid digestibility was determined using both methods. Standardized amino acid digestibility values for the two methods were found to vary among feed ingredients and among samples of the same ingredient. There were generally no differences in amino acid digestibility for six corn and four distillers dried grains with solubles (DDGS) samples between the two

methods. There were greater digestibilities for the PFR for a high protein DDG and a conventionally processed DDGS. The PFR also yielded larger digestibilities for one meat and bone meal sample and a poultry by-product meal, but not for another meat and bone meal.

A third method of determining amino acid digestibility was proposed and developed (Chapter 3). A precision-fed ileal chick assay (PFC) was developed using 3-week-old broilers. Several studies were conducted to determine the length of fasting, amount of feed needed to yield maximal ileal digesta, length of time needed for the undigested feed residues to reach the distal small intestine, and measurement of basal endogenous amino acid flow. These studies indicated that at least eight hours are required to empty the gastrointestinal tract of feed residues and that chicks should be precision-fed approximately 10 g of feed. Four hours post-feeding was adequate to yield maximal amounts of ileal digesta, which is important for amino acid analyses. Chicks were also precision-fed a nitrogen-free diet to estimate ileal endogenous amino acid flow. When compared to previous published results (for chicks ad libitum fed a nitrogen-free diet), the precision-fed chicks yielded higher endogenous amino acid flows.

Several protein sources of both plant and animal origin were obtained and amino acid digestibilities were determined and compared among the three methods, the PFR, SID, and PFC (Chapters 4 and 5). Differences in amino acid digestibility did sometimes occur but these differences were not consistent among methods and ingredients. For corn, the PFC yielded significantly higher values than the PFR and SID. For corn gluten meal, the PFR yielded greater values than the other two methods for the majority of the amino acids. The PFR yielded greater digestibilities than the PFC for all three DDGS samples evaluated, whereas, the PFR values were higher than the SID values for only one of the DDGS samples. Amino acid digestibility values for soybean meal and meat and bone meal were in general agreement for the three methods.

There were some differences among methods for fish meal; however, these differences were not consistent among the methods or amino acids.

Overall, there were no consistent differences among the PFR, SID, and PFC for the 26 samples of feed ingredients evaluated in at least two of the assays. However, the PFR did yield greater amino acid digestibility values than the two ileal chick assays (SID or PFC) for 9 of the 26 samples. As discussed in Chapters 4 and 5, two previous studies by Garcia et al. (2007) and Adedokun et al. (2009) also reported that the values for the PFR were greater than those for the SID for some feed ingredients. These collective results suggest that the PFR may yield greater amino acid digestibility values than the chick ileal assays in some instances. The reason for these differences is unknown, but there are some possible explanations. The differences are probably not due to feeding method since differences were observed between both precision-fed roosters and precision-fed chicks. One possible reason is bird age, with digestibility being greater in roosters because they are older and have a more mature or well developed gastrointestinal tract. However, the effect of age may not be large since previous studies have indicated no difference in amino acid digestibility between 21 day-old chicks and 10-15 day-old chicks, suggesting that the amino acid digestibility is maximized by 21 days or less. Another possible reason for higher values for roosters than chicks is related to where digestibility is measured. In the PFR, digestibility is measured in excreta collected from cecectomized birds; thus, the digesta have passed through the entire small intestine and the very short colon. In the chick ileal assays, digesta is collected from the entire ileal region which represents about one-third of the small intestine. Consequently, some of the amino acids in the collected ileal digesta may have been digested and absorbed if the digesta had been allowed to traverse the entire ileum.

It would also be interesting to know if any differences observed among the assays are repeatable and consistent or if they are due to random variation.

In summary, amino acid digestibility can be determined several different ways. The PFR is a popular and widely used method, but requires the use of surgically modified birds and does not mimic natural feeding behaviors. The SID is also acceptable for estimating ileal amino acid digestibility for younger animals and mimics more natural feeding behaviors but requires larger animal numbers and amount of feed and is more time consuming and expensive. The new PFC has been developed and can provide a rapid method for determining amino acid digestibility of feedstuffs that requires less feed sample and is less expensive than the SID. All three methods seem to be acceptable to determine amino acid digestibility in poultry.

Having three different acceptable methods that are complementary to each other will provide laboratories increased flexibility and, hopefully, will result in increased determination and use of amino acid digestibility in poultry feed formulation in the future. For example, the new PFC can provide a convenient, complementary/supplementary alternative to the SID, particularly when only a few ingredient samples need to be evaluated. Our laboratory routinely determines amino acid digestibility on a large number of feed ingredients annually. Often, only a few samples are received at one time and the results are needed as soon as possible. We also routinely conduct chick growth assays in our research lab each month and not all of the purchased chicks are used. Thus, it would be convenient and efficient to use some of these extra chicks in a PFC rather than order chicks from a commercial hatchery, mix semi-purified diets, and set up a three week experiment to conduct a SID for just a few ingredient samples.

Chapter 7

Curriculum Vitae

Education

- **Doctor of Philosophy** *University of Illinois at Urbana-Champaign, Urbana, IL*
  Department of Animal Sciences
  Research interests: Poultry Nutrition, Advisor: Dr. Carl Parsons
  Dissertation topic: **Development and Evaluation of Different Assays for Determining Amino Acid Digestibility and Metabolizable Energy in Poultry**
  Expected graduation: December 2009
- **Master of Science** *University of Illinois at Urbana-Champaign, Urbana, IL*
  Department of Animal Sciences
  Research interests: Poultry Nutrition, Advisor: Dr. Carl Parsons
  Thesis title: **Nutritional Evaluation of New Corn Dried Distillers Grains with Solubles (DDGS) Produced by New Modified Dry-grind Technologies for Poultry**
  Graduation date: May 2007
- **Bachelor of Science** *University of Illinois at Urbana-Champaign, Urbana, IL*
  Department of Animal Sciences
  Companion Animal, Recreational Animal, and Laboratory Animal degree option
  Graduation date: May 2004

Technical Skills and Abilities-Poultry and Nutrition Specific

- Diet formulation and mixing of both practical and purified experimental diets
- Design and setup of experimental feeding trials for broiler chicks and laying hens
- Measurement of egg components
  - Egg grading, candling, specific gravity procedure, Haugh units, shell quality, and yolk color
- Cecectomized rooster surgery
  - Dosing and administration of anesthesia
  - Practiced in surgery and suture technique
- Experience with precision-fed bioassays for adult roosters and chicks
- Carcass evisceration and collection of meat components
- Collection of excreta and various tissue samples such as blood, tibia, and internal organs
- Bird identification
  - Wing and leg banding
- Hatchery and grower management
  - Egg transfer and incubator set-up
  - Experience in dubbing, dewattling, toe-clipping, and dewclaw removal
  - Vaccinations (aerosol and injection), Fowl pox vaccination, and Pullorum testing

Lab Skills and Abilities

- Nutritional analysis of feed ingredients
  - Dry matter and organic ash
  - ADF, NDF, and ADL analysis
  - Bomb calorimetry and Kjeldahl procedures
- Anaerobic microbial plating techniques

Miscellaneous Skills and Abilities

- SAS, statistical analysis program
- WUFFDA, Feed formulation program
- Microsoft Windows, Microsoft Office, and HTML programming
- Fluent in English and Korean; proficient in Spanish

Collaborative Research Projects

- Supplementation of enzymes and feed additives in poultry nutrition
- Microbial phytase utilization in different production phases for laying hens
- Non-feed withdrawal molting programs
- Nutritional modification of gut microbiology
- Prebiotics in poultry nutrition
- Transgenic corn, soybean meal, and soybean oil in poultry
- Ovarian cancer and flaxseed supplementation in laying hens
- Effect of different broiler strains on carcass composition

Honors and Awards

- Poultry Science Association's Student Research Paper Certificate of Excellence, 2006 and 2007
- International Ingredient Corporation's Pinnacle Award, 2007

Professional Organizations

- Poultry Science Association, 2004-current
- World Poultry Science, 2004-current
- Poultry Science Association's the Hatchery, 2006-current

Teaching experience

- ANSC 205 World Animal Resources-Advanced Composition Spring 2007, 2008, and 2009
- ANSC 103 Domestic Animals in their Environment 2005-2009
- ANSC 100 Introduction to Animal Sciences 2005-2009
- ANSC 404 Poultry Production 2005-2009

- Completed the Teaching College for the College of Agriculture, Consumer and Environmental Sciences, Spring 2007
- Completed Writing Across the Curriculum Graduate Teaching Assistant Training Seminar, Spring 2009

Extracurricular Activities

- Co-founder and Treasurer of the Illini Poultry Club, 2005-2009
- PSA Hatchery Student Champion, 2006-current
- Volunteer with feline rescue group, CatSNAP, 2006-current
- Member of the Parkland Community Orchestra, 2008-2010

Publications

- **Peer Reviewed Journals**

Kim, E.J., R. Srinivasan, V. Singh, and C.M. Parsons. 2010. Nutritional composition, nitrogen-corrected true metabolizable energy, and amino acid digestibilities of new corn distillers dried grains with solubles produced by new fractionation processes. Poultry Science. 89:44-51

Kim, E.J., C. Martinez Amezcua, P. L. Utterback, and C. M. Parsons. 2008. Phosphorus Bioavailability, True Metabolizable Energy, and Amino Acid Digestibilities of High Protein Corn Distillers Dried Grains and Dehydrated Corn Germ**.** Poultry Science. 87: 700-705.

- **Abstracts (first authored)**

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2006. Phosphorus Bioavailability, TME, and amino acid digestibilities of high protein corn distillers dried grains with solubles and dehydrated corn germ meal. Poultry Science. 85:Supplement 1:53

Kim, E.J., C.M. Parsons, V. Singh, and R. Srinivasan. 2007. Nutritional Evaluation of new corn distillers dried grains with solubles (DDGS) produced by the enzymatic milling (E-mill) and Elusieve processes. Poultry Science. 86:Supplement 1: 397

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2008. Development of a precision-fed ileal digestibility assay utilizing 3-week-old broiler chicks. Poultry Science. 87:Supplement 1:31

Kim, E.J., C.M. Jacobs, P.L. Utterback, and C.M. Parsons. 2009. Comparison of amino acid digestibilities using three different methods. Annual Poultry Science Association Annual Meeting Abstract. ACCEPTED

- **Abstracts (co-authored)**

Utterback, P.L., Biggs, P., Martinez, C., **E.J. Kim**, K.W. Koelkebeck, and C.M. Parsons. 2005. Evaluation of limit feeding low-energy diets for a non-feed withdrawal laying hen molt program. Poultry Science. 85: Supplement 1:68

Biggs,P., P.L. Utterback, **E.J. Kim**, C.M. King, R.N. Dilger, C. Scherer, and C.M. Parsons, The effects of whole grains on growth performance, nutrient digestibilities, and cecal short-chain fatty acid production in young chicks. Poultry Science. 86:Supplement 1:52.

Utterback, P.L., **E. J. Kim**, C. M. King, K. W. Koelkebeck, and C. M. Parsons. 2006. Evaluation of limit feeding low-energy diets for a varying number of days in a non-feed withdrawal laying hen molt program. Poultry Science 86:Supplement 1:131

Utterback, P.L., **E.J. Kim**, C. Jacobs, C. Utterback, C. Parsons, J. Snow, and J. Weigel. 2007. Evaluation of NutriDense® corn compared to conventional corn fed to laying hens. Poultry Science 86:Supplement 1:70