Effects of Diet Type and Ingredient Composition on Rate of Passage and Use of In Vitro Assays to Predict Amino Acid Digestibility of Animal Protein Meals in Broilers

by

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ABSTRACT

Ileal amino acid digestibility (IAAD) is used to characterize amino acid (AA) quality of animal protein meals (APM) for poultry, and IAAD assays utilize semi-purified (SP) diets. Nutrient utilization is influenced by rate of passage (ROP), and it has not been determined if differences exist in ROP between corn soybean-meal diets and semi-purified diets in IAAD assays. The IAAD assay is laborious and costly, and *in vitro* assays to predict IAAD of APM commonly used in the poultry industry would be useful tools for nutritionists.

The first experiment evaluated the effects of diet type and ingredient composition on rate of passage (ROP) and apparent IAAD in broilers from 14 to 22 d of age. Experimental diets were formulated to contain 20% CP and consisted of: 1) corn-soybean meal-based (CSM) diet containing porcine meat and bone meal (MBM) (5% inclusion), 2) CSM diet containing distiller's dried grains with solubles (DDGS) (5% inclusion), 3) SP diet containing MBM (38% inclusion), and 4) SP diet containing DDGS (76% inclusion). Time of 50% TiO_2 excretion (T50) and mean retention time (MRT) indicated a faster ($P < 0.05$) ROP for SP-DDGS than the 2 CSM diets. There were no differences between T50 or MRT of SP-MBM and CSM-MBM. In general, apparent IAAD values were higher ($P < 0.05$) for the 2 CSM diets than for SP diets.

The second experiment evaluated a novel digestive enzyme assay (Poultry Complete IDEA, Novus International, Inc., St. Charles, MO) (PC IDEA) and the pepsin

digestibility assay as predictors of standardized IAAD of 20 APM fed to broilers from 25 to 30 d of age. Standardized IAAD, pepsin digestibility, and PC IDEA predicted digestibility were determined for 10 meat and bone meals and 10 animal protein blends. Pepsin digestibility and PC IDEA were both significantly correlated ($P < 0.001$) with SIAAD. Prediction equations for SIAAD of Lys, Met, and Thr were: % Lys SIAAD = [–9.65 + (0.38 × % PC IDEA predicted Lys digestibility) + (0.69 × % pepsin digestibility)], % Met SIAAD = [–35.95 + (0.62 × % PC IDEA predicted Met digestibility) + (0.75 × % pepsin digestibility)], % Thr SIAAD = [–77.5 + (0.39 × % PC IDEA predicted Thr digestibility) + (1.37 × % pepsin digestibility)]. Values of R^2 for Lys, Met, Thr, Val, and Ile were 0.46, 0.47, 0.55, 0.51, and 0.49, respectively.

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I. INTRODUCTION

Approximately 50 billion pounds of inedible by-products are generated in the United States annually from the processing of animals produced for meat, milk, eggs, and fiber (Meeker and Hamilton, 2006). Inedible by-products undergo chemical and physical processes of rendering to yield fats, oils, and animal protein meals (**APM**) that are suitable for animal feeds. Animal protein meals can be excellent sources of dietary amino acids (**AA**), calcium, and phosphorous for poultry. Meat and bone meal (**MBM**) is the rendered product from mammalian tissues, while poultry by-product meal (**PBM**) consists of ground and rendered carcass parts of slaughtered poultry. In the past, MBM and PBM were the primary APM used in poultry diets. Currently, by-products of poultry processing are segregated for the production of pet-food grade and feed-grade PBM. The former consists of highly digestible parts and is marketed at a premium price, while the latter contains higher proportions of poorly digested parts and is more variable in nutrient quality. Consequently, nutritionists are including more MBM in poultry diets. However, due to inconsistencies in raw materials and processing techniques, nutrient composition of MBM varies substantially among commercial sources. Therefore, commercial blenders may combine APM sources such as MBM, PBM, fish meal, feather meal, and supplemental AA in attempt to formulate products that are less variable in AA quality, and these products are classified as animal protein blends.

Large variability has been reported for AA digestibility values of APM fed to poultry. Previous studies have primarily been limited to MBM and PBM and have not included animal protein blends representative of APM currently used in the commercial poultry industry. Furthermore, most previous research has been based on excreta assays. However, ileal AA digestibility (**IAAD**) values of feed ingredients in diet formulations are gaining popularity worldwide.

In the IAAD assay, the test ingredient is included in a semi-purified diet. Diet composition influences rate of passage (**ROP**), which in turn influences nutrient utilization. It has been determined that nutrient utilization of distillers dried grains with solubles (**DDGS**) differs when included in a semi-purified diet vs. a corn soybean-meal diets (Adeola and Ileleji, 2009). However, it has not been determined if differences exist in ROP between corn soybean-meal diets and semi-purified diets in AA digestibility assays.

In vitro digestibility techniques have been developed as rapid assessments of the AA quality of APM. Such assays usually simulate 1 or more steps of *in vivo* digestion. The pepsin digestibility assay has been used to differentiate between high and low quality APM, but provides little value for predictions of AA digestibility that could be utilized in diet formulations (Ravindran and Bryden, 1999). Prediction equations would help nutritionists avoid over or under estimating digestible AA contents of APM and more accurately formulate diets. A novel digestive enzyme assay has been developed to predict the AA digestibility of APM for poultry. Currently, no research has been conducted on the relationship between the novel digestive enzyme assay and IAAD of APM.

II. LITERATURE REVIEW

AMINO ACID AVAILABILITY AND DIGESTIBILITY

Although used interchangeably, AA availability and digestibility are not synonymous terms. Amino acids are biologically available only if they can be digested, absorbed, and utilized by the animal in structural or metabolic proteins, enzymes, or as precursors of body components (Batterham, 1992). The bioavailability of an AA in a feed ingredient can be determined using the slope-ratio technique. In this approach, an animal is fed increasing concentrations of the AA supplied by graded additions of the test ingredient in a basal diet deficient in the AA of interest, and a biological response is measured (Batterham, 1992). The response to increasing AA concentrations from the test ingredient is compared with a standard source. This procedure is laborious and costly because only 1 AA can be measured per assay (Ravindran and Bryden, 1999). Digestibility is defined as the fraction of an ingested nutrient that is absorbed by the bird and not excreted in the feces (Lemme et al., 2004). While digestibility does not directly indicate AA utilization, it is considered the greatest limiting factor of AA availability. Digestibility of all 23 AA can be determined in a single balance assay (Batterham, 1992). Hence, AA digestibility is accepted as the preferred indicator of AA availability in practical feed formulation (Ravindran and Bryden, 1999).

DIET FORMULATION BASED ON DIGESTIBLE AMINO ACIDS

The goal of diet formulation is to provide nutrients that meet maintenance and production requirements of the bird in an economical and sustainable manner (Lemme et al., 2004). In the past, poultry diets were formulated based on the total AA concentrations of feed ingredients. However, formulating diets on a digestible AA basis increases accuracy, minimizes nutrient excesses, and reduces costly safety margins, especially for ingredients that are variable in AA composition (Rostagno et al., 1995; Dari et al., 2005). Wang and Parsons (1998a) reported birds fed diets containing 10 or 20% MBM and formulated on a total AA basis showed reduced growth and efficiency compared with those fed corn-soybean meal diets. When diets were formulated on a digestible AA basis, performance of birds fed MBM-containing diets was similar to birds fed corn soybean-meal diets. The use of digestibility AA values also enables diet formulation based on digestible AA ratios, which allows AA requirements to be met without placing a minimum specification for CP (Emmert and Baker, 1997). Diets that are reduced in crude protein and formulated with supplemental AA require less intact protein sources, ultimately lowering diet cost and reducing nitrogen excretion (Corzo et al., 2005). Thus, broiler diet formulation on a digestible basis can lead to substantial economic and environmental benefits (Rostagno et al., 1995; Dari et al., 2005).

BIOASSAYS TO DETERMINE AMINO ACID DIGESTIBILITY

Accurate AA digestibility values of feed ingredients are required to formulate diets on a digestible AA basis. Balance assays to determine AA digestibility are based on the subtraction of AA recovered in excreta or ileal digesta from the quantity of AA ingested by the animal. However, in addition to undigested dietary AA, excreta and ileal

digesta contain AA of endogenous origin. Estimates that are not corrected for endogenous AA may be underestimated and are termed "apparent" digestibility values. Consequently, correction of AA digestibility coefficients for endogenous AA is preferred (Ravindran and Bryden, 1999).

Endogenous Amino Acid Flow and Methods for Correction

Endogenous AA originate from digestive secretions such as saliva, bile, gastric, pancreatic, and intestinal secretions, as well as mucoproteins, sloughed epithelial cells, serum albumin, and amides (Nyachoti et al., 1997; Ravindran and Bryden, 1999; Adedokun et al., 2011). In chickens, the most predominant endogenous AA are glutamic and aspartic acids, serine, and threonine (Angkanaporn et al., 1997; Adedokun et al., 2007; Golian et al., 2008). Endogenous AA can be divided into basal and diet-specific losses. Basal endogenous AA losses are directly related to dry matter intake and independent of dietary composition. Diet-specific losses stem from the many dietary components that may influence endogenous AA secretion, including AA concentration, fiber type and content, phytate content, and anti-nutritional compounds (Adedokun et al., 2011).

The terminology used in describing AA digestibility methods has been inconsistent. It has been proposed that AA digestibility values corrected for endogenous losses be expressed as "true" or "standardized", depending on which measurements of endogenous AA are considered in digestibility calculations (Stein et al., 2007). In the past, "true" AA digestibility was commonly used to specifically refer to AA digestibility values determined with the precision-fed rooster assay. More recently, the term true AA digestibility is used to define values that have been corrected for both basal and diet-

specific endogenous losses in AA outflow (Stein et al., 2007; Adedokun et al., 2011). True AA digestibility values of an ingredient can be determined with the regression method by feeding graded concentrations of the ingredient and extrapolating AA outflow at zero AA intake (Short et al., 1999; Rodehutscord et al., 2004). However, this method may not be appropriate for practical feed formulation as it requires technical complexity to calculate AA digestibility coefficients, and extrapolation may be subject to large estimation errors (Lemme et al., 2004).

Values for "standardized" AA digestibility are determined by subtracting basal endogenous losses from AA outflow, without regard to diet-specific losses (Lemme et al., 2004; Stein, 2007; Adedokun, 2011). Basal endogenous AA output can be determined from the AA analysis of excreta collected from fasted birds. However, this method is criticized because fasting creates an abnormal physiological state in bird. Furthermore, endogenous losses in this method are not stimulated by dry matter intake (Butts et al., 1993; Lemme et al., 2004). Basal endogenous AA have also been measured with purified diets containing enzymatically-hydrolyzed casein, which is assumed to be nearly 100% digestible. Undigested dietary protein (free AA and small peptides) from casein can be separated from larger endogenous protein based on size (Ravindran and Bryden 1999). Techniques involving isotope markers and guanidinated Lys have also been used to distinguish dietary AA of protein-containing diets from endogenous AA. One advantage of feeding protein-containing diets is the stimulation of proteolytic and pancreatic enzymes, which are significant components of endogenous AA. However, these methods require more extensive analysis of AA outflow, and may lead to error in the separation of endogenous AA from undigested dietary AA (Ravindran and Bryden, 1999; Lemme et

al., 2004). Another strategy involves feeding a nitrogen-free diet. Nitrogen-free diets stimulate endogenous AA flow related to dry matter intake, and eliminate the need to separate dietary and endogenous AA in excreta or ileal digesta (Adedokun et al, 2007). Nitrogen-free diets usually consist of a mixture of purified carbohydrates (corn starch, sucrose, or dextrose), powdered cellulose, supplemental fat, vitamins, and minerals. It has been reported that determination of basal endogenous AA flow with nitrogen-free diets is reliable and reasonably consistent across laboratories (Adedokun et al., 2007).

Precision-Fed Rooster Assay

Many of the published AA digestibility values for poultry feedstuffs are based on the precision-fed rooster assay developed by Sibbald (1976). This assay involves fasting adult Single Comb White Leghorn roosters for 24 to 48 h, placing a known quantity of test ingredient directly into the crop via intubation, and quantitatively collecting excreta for 48 h. This assay is widely accepted and popular because it is relatively rapid and simple, requires only a small amount of the test ingredient, and avoids palatability issues. Also, many feed ingredients can be assayed simultaneously using multiple roosters and the roosters can be kept for numerous assays (Ravindran and Bryden, 1999; Lemme et al., 2004).

The precision-fed rooster assay is not without limitations. Amino acids of urinary origin are present in the excreta as poultry excrete feces and uric acid together. It is often assumed that concentrations of AA in urine are small and have negligible effects on excreta digestibility estimates (Sibbald, 1987). However, this assumption has been questioned, and it has been suggested that over-processed ingredients may lead to increased excretion of AA as metabolites in the urine (Fernandez and Parsons, 1996;

Angkanaporn et al., 1997). Furthermore, the majority of anaerobic microorganisms in the poultry gastrointestinal tract are concentrated in the ceca, which are 2 elongated pouches located in the hindgut. Microbial proteolysis, deamination of AA, and retention of endogenous AA in the ceca can influence AA digestibility estimates, and it has been reported that microbial protein may contribute as much as 25% of total excreta protein (Parsons et al., 1982; Ravindran and Bryden, 1999). If the net result of microbial activity is protein degradation, excreta values will overestimate AA digestibility (Ravindran et al., 1999). To eliminate the influence of hindgut modification, the precision-fed rooster assay is conducted with cecectomized (**CEC**) roosters. Parsons (1986) determined the AA digestibility of MBM in CEC roosters averaged 10% units lower than with intact roosters. Additionally, Lys digestibility determined in CEC roosters was in good agreement with Lys bioavailability determined with the slope-ratio assay (Parsons, 1986).

Ileal Amino Acid Digestibility Assay

A majority of the uptake of AA occurs in the small intestine prior to the terminal ileum (Webb, 1990). Payne et al. (1968) suggested the collection of ileal digesta in digestibility assays to circumvent the influence of urinary AA and microbial modification of AA in the hindgut. Ileal digesta can be collected by inserting a cannula at the terminal ileum, or by sacrificing birds and extracting the small intestine. Ileal cannulation is a complex surgical procedure, and obtaining a sufficient amount of digesta with this technique is difficult (Ravindran and Bryden, 1999). Thus, extraction of the ileum is preferred, and this has led to the development of the IAAD assay.

A major advantage of the IAAD assay is that it can be conducted with growing birds. The test ingredient is the only source of AA in a semi-purified diet, and AA

digestibility coefficients of the diet are assumed to be representative of AA digestibility of the test ingredient (Ravindran and Bryden, 1999). Because ileal digesta cannot be quantitatively collected, a dietary marker is included in the diet to allow indirect determination of IAAD. Birds are provided test diets 5 to 7 d prior to ileal collection to allow for intestinal adaptation to the semi-purified diet. Following adaptation, birds are euthanized and ileal contents are collected by gently flushing with deionized water. It has been suggested that collection from only the latter two-thirds of the ileum to 2 cm proximal to the ileo-cecal junction is preferred (Kluth et al., 2005). Apparent IAAD coefficients are determined by the ratio of the concentration of the marker in the diet to the concentration in ileal digesta (Lemme et al., 2004). Apparent coefficients can be standardized for basal endogenous AA values determined in the same study using N-free or high digestible protein diets.

A critical assumption of the IAAD assay is that the dietary marker is not altered, digested, or absorbed in the gastrointestinal tract, and that flow rates of the marker and digesta are similar. Furthermore, the marker must be homogeneously mixed in the diet and have a high recovery rate in the digesta. Acid-insoluble ash, chromic oxide, and titanium dioxide are commonly used dietary markers in poultry nutrition studies (Sales and Janssens, 2003). Acid-insouble ash has been the most widely-used marker for excreta-based metabolizability and digestibility studies; however, a large sample size (~3 g) is required for accurate determination and limits its use for ileal digesta analysis (Scott and Boldaji, 1997). Chromic oxide can be added to the diet at a low inclusion rate (0.25-0.50%) and mixed homogenously. The green color of chromic oxide is transferred to the diet and can be observed in excreta, and this is advantageous if excretion needs to be

visually assessed. Spectrophotometry can be used to measure chromic oxide, and only a small amount of sample is required (Sales and Janssens, 2003). Sibbald et al. (1960) suggested digestibility data using chromic oxide may lead to greater precision than the total collection method for metabolizable energy studies. On the other hand, additional reports on the reliability of chromic oxide have been varied (Sales and Janssens, 2003). Also, analysis of chromic oxide involves the use of strong oxidizing agents such as perchloric acid, which requires a specialized fume hood.

Titanium dioxide is a white powder that is virtually odorless and tasteless (Peddie et al., 1982). Similar to chromic oxide, titanium dioxide can be homogenously mixed in the diet at a relatively low inclusion level (0.5%) and has been reported to be a viable marker for digestibility studies in rats (Krawielitzki et al., 1987), pigs (Jagger et al., 1992), and chickens (Peddie et al., 1982; Short et al., 1996). Titanium dioxide concentration of digesta or excreta is determined in a small sample (~ 0.25 g) that is ashed and dissolved in sulphuric acid, digested on a heating block, and diluted with deionized water (Short et al., 1996). Hydrogen peroxide is added to an aliquot of the sample and a very sensitive reaction occurs that results in a stable orange/yellow color (Short et al., 1996). Absorbance is measured using a UV spectrophotometer at 410 nm (Short et al., 1996). Peddie et al. (1982) reported less variation in dry matter digestibility of layers when using titanium dioxide as a marker than with the total excreta collection method, and overall titanium dioxide recovery was 97.5%.

Effects of Collection Method and Age on Amino Acid Digestibility Values

Inconsistent differences between excreta and ileal AA digestibility have been reported (ten Doeschate et al., 1993; Ravindran et al., 1999; Kadim et al., 2002).

Ravindran et al. (1999) observed no consistent pattern in magnitude or direction between ileal or excreta estimates of AA digestibility for 12 feed ingredients in 5-week old male broilers. Kadim et al. (2002) reported AA digestibility of 6 ingredients fed to broilers were from 8 to 56% higher with excreta collection than with ileal digesta collection. Therefore, ileal and excreta AA digestibility values are inconsistent, and ileal digestibility may provide a more accurate assessment of AA digestibility (Ravindran et al., 1999; Kadim et al., 2002; Lemme et al., 2004).

The combined effects of age, collection method, and bird type have been evaluated by comparing digestibility values for a range of ingredients determined with the precision-fed CEC rooster assay and the IAAD assay. Garcia et al. (2007) reported that AA digestibility determined in precision-fed CEC roosters was significantly higher than standardized IAAD in 7 d old broilers for corn, wheat, soybean meal, poultry by-product meal, feather meal, and fish meal. Amino acid digestibility in roosters also tended to be higher than in 21 d old broilers, but differences were less consistent. Likewise, Adedokun et al. (2009) observed higher values with the precision-fed CEC rooster assay than with the standardized IAAD assay in broilers for 3 sources of DDGS, whereas significant differences were not observed for canola meal, soybean meal, or meat and bone meal. Kim et al. (2011) determined AA digestibility of corn (6), DDGS (6), MBM (2), and PBM (2) with the precision-fed CEC roosters and the standardized IAAD assay. No significant differences were observed in AA digestibility between methods for the corn samples. However, AA digestibility was higher with the precision-fed CEC assay than the IAAD assay for 2 of the DDGS and 1 of the MBM, but lower for the other 2 MBM. Consequently, the validity of AA digestibility determined with mature, Single

Comb White Leghorn roosters in the precision-fed rooster assay for growing broiler chicks is often questioned.

The precision-fed CEC rooster assay and the IAAD assay are both acceptable methods for the determination of AA digestibility. The precision-fed rooster assay may over-estimate AA digestibility, especially for APM. However, differences between the 2 methods are inconsistent. The precision-fed CEC rooster assay may be desirable for routine or rapid evaluation of feed ingredients, but the IAAD allows ad libitum feeding, avoids hindgut modification, and provides AA digestibility estimates determined in the desired bird type and age. Thus, the IAAD assay is gaining acceptance as the preferred method for determining AA digestibility of feed ingredients (Bryden and Li, 2010).

RATE OF FEED PASSAGE

The rate of feed passage through the gastrointestinal tract influences nutrient utilization by determining the time available for nutrient interaction with digestive enzymes, absorptive surfaces, and microbial populations (Rao and Clandinin, 1970; Mateos and Sell, 1980). As a result, longer retention of ingredients in the gastrointestinal tract may increase nutrient utilization. Rate of passage is influenced by multiple dietary factors such as particle size (Svihus et al., 2002), fiber type (Almirall and Esteve-Garcia, 1994; Lázaro et al., 2003; Hetland et al., 2004), concentration of supplemental fat (Mateos et al., 1982; Dänicke et al., 1999), or carbohydrate source (Mateos and Sell, 1981; Weurding et al., 2001). Mateos and Sell (1981) suggested that the decrease in rate of passage with increasing concentrations of supplemental fat may be responsible for the "extrametabolic" effect of fat additions in poultry diets.

The influence of diet composition on ROP has significant implications for bioassays. Nutrient utilization of test ingredients may differ when fed as single ingredients, in semi-purified diets, or in practical diets due to differences in ROP. Adeola and Ileleji (2009) determined the energy value of DDGS as 2,963 kcal ME_n/kg when using a SP diet, while a lower value of 2,787 kcal ME_n/kg was observed when using a corn-soybean meal-based basal diet. The discrepancy in the determined energy values of the DDGS may have been partly attributed to differences in ROP between the semi-purified and corn-soybean meal basal diet.

Rate of passage is also influenced by numerous husbandry and experimental variables. These include strain and age of the bird (Shires et al., 1987; Washburn, 1991), lighting schedule (Buyse et al., 1993), ambient temperature (Wilson et al., 1980), feed withdrawal or fasting period (Mateos et al., 1982), and marker type (Ferrando et al., 1987; Vergara et al., 1989; Washburn, 1991). A course marker substance may be retained in the gizzard, while a soluble marker may pass through the gastrointestinal tract more rapidly than digesta (Vergara et al., 1989). For the reasons previously discussed for the IAAD assay, chromic oxide and titanium dioxide appear to be suitable markers for the determination of rate of passage.

A number of measurements have been used by researchers to assess rate of passage. Much research has been based on time until visual first appearance of a marker substance in the excreta, or first appearance of excreta following a fasting period (Hillerman et al., 1953; Sibbald, 1979; Mateos et al., 1982). Ferrando et al. (1987) suggested using cumulative marker excretion curves to determine the time required to excrete a specified proportion of ingested marker, such as 1% (**T1**) or 50% (**T50**). Also,

Coombe and Kay (1965) used cumulative excretion curves to calculate mean retention time. Mean retention time, T1, and T50 are more precise and less subjective measurements than visual first appearance, and have been demonstrated as appropriate measurements of rate of passage (Vergara et al., 1989; Almirall and Esteve-Garcia, 1994, Svihus et al., 2002).

AMINO ACID DIGESTIBILITY OF ANIMAL PROTEIN MEALS

Substantial variability in digestible AA content is a limiting factor for high inclusion levels of APM in broilers diets (Parsons et al., 1997). Kim et al. (2011) reported the AA concentrations of 2 samples of MBM to be 2.21 and 1.92% for Lys, 0.60 and 0.42% for Met, and 1.46 and 1.07% for Thr. Standardized ileal amino acid digestibility was 47.4 and 76.15% for Lys, 45.6 and 75.6% for Met, and 48.0 and 69.7% for Thr. Ravindran et al. (2002) determined apparent IAAD of 19 MBM samples produced in New Zealand to be 68.3% for Lys, 72.7% for Met, and 59.5% for Thr, with ranges of 38.5, 36.5, and 35.4 percentage points, respectively (Ravindran et al., 2002). The greatest variability in apparent IAAD was observed for Cys, which ranged from 14.8 to 56.3% with an average of 36.5% and a coefficient of variation of 28.3%. The variability of Cys digestibility is important as it is the first limiting AA in MBM for broilers (Wang and Parsons, 1997).

Raw material source has a substantial influence on AA digestibility. Wang and Parsons (1998b) reported that AA digestibility of MBM produced from mixed species was generally lower than bovine or porcine MBM. Skurray and Herbert (1974) determined the nutritive value for MBM containing high proportions of bones and connectives tissues was lower than MBM produced from soft offal. The protein content

of bones and connective tissues is largely comprised of collagen (Eastoe and Long, 1960). Collagen is deficient in most essential AA and abundant in nonessential AA, such as Pro, Gly, and hydroxyproline (Eastoe and Long, 1960; Shirley and Parsons, 2001). High proportions of bone in MBM are indicated by high ash contents. Negative influences of ash content on AA digestibility of MBM have been reported (Karakas et al., 2001; Ravindran et al., 2002). However, Shirley and Parsons (2001) determined the detrimental effects of ash on AA quality of MBM were primarily due to lower concentrations of essential AA, and not reduced AA digestibility.

Animal protein meals are produced using a variety of processing systems and temperatures. Batch and continuous-flow cooking systems are commonly used with steam cooking temperatures ranging from 115 to 145ºC (Meeker and Hamilton, 2006). Configuration of processing system and processing temperature both influence AA digestibility of APM (Skurray and Herbet, 1974; Wang and Parsons, 1998b). The influence of processing temperature on AA digestibility has varied, although it is generally recognized that higher temperatures reduce AA digestibility of APM (Wang and Parsons, 1998b). The most pronounced detrimental effect of increased processing temperature on AA digestibility was observed for Cys (Wang and Parsons, 1998b).

IN VITRO PREDCTION OF AMINO ACID DIGESTIBILITY

Prediction of AA digestibility of feedstuffs using *in vitro* assays is an attractive strategy because such assays are simple, economical, and rapid when compared with bioassays. Enzymatic assays involve incubation of feed ingredients with 1 or more proteolytic enzymes under controlled conditions, and estimating the extent of digestion. Current single enzyme assays may be sufficient for identification of severely heat-

damaged sources or for relative rankings of APM but provide little use for accurate prediction of AA digestibility (Ravindran and Bryden, 1999).

The pepsin digestibility assay has been widely accepted by the feed industry for assessing protein quality of APM (Ravindran and Bryden, 1999). In this assay, ground samples are fat extracted with ether, combined with pepsin solution (0.2% pepsin) and agitated for 16 h, and the resulting digested solution is filtered (AOAC, 1995). Crude protein content of the indigestible residue remaining on the filter is determined using the Kjeldahl method, and results are expressed as percent of CP digested by pepsin (AOAC, 1995). With sufficient equipment, many samples can be run simultaneously in this assay.

The pepsin concentration recommended by the AOAC (1995) has been questioned by researchers. It was suggested by Johnston and Coon (1979) that a pepsin concentration of 0.2% was too concentrated and did not allow adequate differentiation between MBM sources. Reducing the pepsin concentration to 0.02 or 0.002% improved the sensitivity of the assay. Similarly, Parsons et al. (1997) did not observe significant correlations of 0.2% pepsin digestibility and Lys digestibility in CONV or CEC precision fed roosters. However, significant correlations of 0.62 and 0.69, respectively, were observed when based on pepsin digestibility with a 0.002% pepsin concentration. Lysine bioavailability and pepsin digestibility were also significantly correlated. More recently, Ravindran et al. (2002) did not detect any significant correlations between pepsin digestibility (0.2% pepsin) and apparent IAAD digestibility of 19 MBM in 5 wk old broilers, except for Gly, which was negatively correlated with a coefficient of -0.51.

The immobilized digestive enzyme assay (IDEA) was developed as a technique to determine protein digestibility of human foodstuffs (Porter et al. 1984; Chang et al.,

1990). In the original IDEA assay, samples were initially digested with immobilized pepsin, and subsequently digested with immobilized trypsin, chymotrypsin, and intestinal peptidases (Porter et al. 1984). Protein digestion is quantified by the reaction of liberated α-amino groups with o-phthaldialdehyde (OPA), which can be measured spectrophotometrically. Immobilized enzymes can be separated from the reaction mixture and used again as active enzymes in subsequent reactions (Porter et al., 1984). Additional advantages of immobilized enzymes include increased stability over a broader range of pH and temperature, and less enzyme contamination from waste streams (Porter et al., 1984).

The original IDEA assay was modified by Schasteen et al. (2007) to provide more rapid prediction of AA digestibility for soybean meal. The modified system eliminated the initial pepsin digestion step, and reduced the time required for the assay from ~2.5 d to ~1 d. The soybean meal IDEA system was reported to be an excellent predictor of standardized AA digestibility determined in precision-fed roosters with R^2 values of 0.86, 0.88, 0.88, 0.86, and 0.90 for Lys, Met, Thr, Val, and Ile, respectively (Schasteen et al. 2007).

An assay similar to soybean meal IDEA has been developed to determine the AA digestibility of APM. A single digestive enzyme is used in place of the immobilized enzymes, and the assay takes approximately 4 h to complete. Boucher et al. (2009) compared AA digestibility coefficients predicted with the novel assay for fish meal (n = 5) with *in vivo* digestibility determined in precision-fed roosters. *In vitro* predicted values were highly correlated with *in vivo* values, and linear regression resulted in values of R^2 that ranged from 0.73 for Lys to 0.99 for Arg. Therefore, much of the variability in

AA digestibility of highly digestible fish meal was explained by the novel digestive enzyme assay, but relationships with other APM currently used by the poultry industry have not been reported.

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III. EFFECTS OF DIET TYPE AND INGREDIENT COMPOSITION ON RATE OF PASSAGE AND APPARENT ILEAL AMINO ACID DIGESTIBILITY IN BROILER CHICKS

ABSTRACT

This experiment evaluated rate of passage (ROP) and apparent ileal amino acid digestibility (AIAAD) of 4 diets varying in ingredient composition fed to broilers from 14 to 22 d of age. Two hundred and eighty-eight Ross × Ross 708 chicks (12 birds per cage; 0.45 m^2 per bird) were randomly assigned to 24 cage (6 replicate cages per treatment) at 1 d of age. Experimental diets were: 1) corn-soybean meal-based (CSM) diet containing porcine meat and bone meal (MBM) (5% inclusion), 2) CSM diet containing distiller's dried grains with solubles (DDGS) (5% inclusion), 3) semi-purified (SP) diet containing MBM (38% inclusion), and 4) SP diet containing DDGS (76% inclusion). Diets were formulated to contain 20% CP and were adequate for vitamins and minerals. Experimental diets were provided from d 14 to 22. On d 18, a total excreta collection was conducted every h for 12 h from 8:00 to 19:00 h to determine ROP, and AIAAD was determined on d 22. Time of 1% TiO_2 excretion (T1), 50% TiO_2 excretion (T50), and mean retention time (MRT) were used to express ROP. The SP-MBM diet reached T1 ($P < 0.05$) faster than the SP-DDGS or 2 CSM diets. However, T50 indicated a faster ($P < 0.05$) ROP for SP-DDGS than the 2 CSM diets, and no significant difference was observed between the 2 SP diets. The MRT of the SP-DDGS diet (5.13 h) was less ($P < 0.05$) than the MRT of the SP-MBM, CSM-MBM, and CSM-DDGS diets, which resulted in values of 5.48, 5.62 and 5.58 h, respectively. In general, the AIAAD values

were higher ($P < 0.05$) for the 2 CSM diets than for SP diets. Except for His, no statistical differences were observed between the AIAAD of the 2 CSM diets. Comparing the 2 SP diets, AIAAD was usually similar or higher ($P < 0.05$) for SP-DDGS, except for His, Lys, and Gly, which were higher ($P < 0.05$) for SP-MBM. Based on T50 and MRT, semi-purified diets containing DDGS had a faster ROP in broilers than CSM diets or SP diets containing MBM.

INTRODUCTION

Nutrient utilization of feed ingredients in broilers is a result of the physiological response of the bird to the physicochemical properties of the ingredient (McNab, 1994). The rate of feed passage (**ROP**) through the gastrointestinal tract influences nutrient utilization by determining the time available for nutrient interaction with digestive enzymes, absorptive surfaces, and microbial populations (Rao and Clandinin, 1970; Mateos and Sell, 1980). The ROP of a feed ingredient may be influenced by fiber type (Almirall and Esteve-Garcia, 1994; Lázaro et al., 2003; Hetland et al., 2004), inclusion of supplemental fat (Mateos et al., 1982; Dänicke et al., 1999), or carbohydrate source (Mateos and Sell, 1981; Weurding et al., 2001).

Shires et al. (1987) determined birds fed corn meal-based diets containing 37% soybean meal had a longer mean retention time (**MRT**) than those fed diets with the same inclusion of canola meal. These authors suggested the fiber content of the canola meal may have contributed to a shorter MRT, while the oligosaccharide content of the soybean meal may have led to longer retention in the ceca. Meat and bone meal (**MBM)** and distiller's dried grains with solubles (**DDGS)** are 2 co-products commonly used as protein

sources in poultry diets. Rate of passage has not been reported with diets containing MBM or DDGS.

Bioassays employed to evaluate amino acid digestibility of feed ingredients often utilize semi-purified diets (**SP**). However, estimates of nutrient or energy utilization of feed ingredients determined with SP diets may not be consistent with the actual nutrient or energy contribution of those ingredients when included in practical diets. Using the regression method, Adeola and Ileleji (2009) determined the energy value of corn DDGS as 2,963 kcal ME_n/kg when using a SP diet, while a lower value of 2,787 kcal ME_n/kg was observed when using a corn-soybean meal-based (**CSM**) basal diet. Although it is likely that multiple factors were responsible for the discrepancy in the determined energy values, differences in the ROP of the 2 diet types may have been a determinant.
The question remains as to whether differences in ROP of diet types may correspond to modification of nutrient utilization of feed ingredients, and ultimately, broiler performance. The objective of this experiment was to examine the ROP of SP and CSM diets containing MBM or DDGS. In addition, the apparent ileal amino acid digestibility (**AIAAD**) of the experimental diets was determined as a point of reference to examine differences in nutrient utilization between the experimental diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the experimental protocol involving live birds (PRN 2010-1716).

Bird Husbandry

Two-hundred and eighty-eight Ross × Ross 708 (Aviagen, Inc., Huntsville, AL) male chicks were obtained from a commercial hatchery, and received vaccines for

Marek's disease, Newcastle disease, and infectious bronchitis. Chicks were placed into grower battery cages (Petersime**,** Gettysburg, OH) at 12 birds per cage, and each cage (68 cm × 68 cm × 38 cm) was equipped with 1 trough feeder and 1 trough waterer. The battery cages were located in a solid-sided house with temperature control. Temperature was set at 33^{o}C at placement and was decreased gradually to 27^{o}C by the end of experimentation. A 23L:1D lighting schedule was used until d 18. On d 19, a 12L:12D lighting schedule was implemented to obtain sufficient feed intake approximately 3 h prior to collection to ensure that adequate amounts of ileal digesta could be collected on d 22. Broilers were fed a common corn-soybean meal starter diet (AME_n, 3,075 kcal/kg; digestible Lys, 1.22%; digestible TSAA, 0.92%; digestible Thr, 0.83%; Ca, 0.90%; and non-phytate P, 0.45%) until receiving experimental diets at 14 d of age.

Dietary Treatments

Four dietary treatments consisted of 2 CSM diets containing porcine MBM (5% inclusion) or DDGS (5% inclusion), and 2 SP diets containing MBM (38% inclusion) or DDGS (76% inclusion). Experimental diets were formulated to contain 20% CP, which required varying levels of MBM and DDGS in SP diets. All diets met or exceeded NRC (1994) nutrient recommendations. Two sets of experimental diets were mixed that were identical in ingredient and nutrient composition with the exception of an inert marker. One set of experimental diets contained no marker, while TiO_2 was added (0.5%) to the other set of experimental diets as an inert marker. All experimental diets were provided in mash form. Acid detergent fiber (method 973.18 (a-d), AOAC, 2006) and neutral detergent fiber (Van Soest et al., 1991) of the experimental diets were determined by the University of Missouri Agricultural Experiment Station Chemical Laboratory. Crude fat

contents of the experimental diets were determined using hexane extraction-submersion (method 2003.06, AOAC, 2006).

Determination of Rate of Passage

Experimental diets without TiO_2 were provided at 14 d of age. Following a 4 day acclimation period, an assay was conducted to determine ROP on d 18. A 23L:1D lighting schedule was used through d 18 with light provided from 06:00 to 05:00 h. The assay was initiated with a 2 h fasting period beginning at 05:00 h (Svihus et al., 2002). Pans under the battery cages were cleaned prior to placement of diets containing TiO_2. At 07:00 h, access to experimental diets containing TiO_2 was provided for 2 h. Diets were replaced with experimental diets without TiO_2 at 9:00 h to allow *ad libitum* feed intake for the remainder of the ROP assay. Consumption of diets containing TiO_2 was used to determine total TiO_2 intake. Beginning at 08:00 h, total excreta collections were conducted each h for 12 h, where all excreta from each pen were collected, weighed, and frozen until later analysis.

Frozen excreta samples were thawed, lyophilized, and ground using an electric coffee grinder due to the small sample size (~7.5 g, dry wt.) of the collected excreta to provide a finely ground sample while avoiding significant loss. Titanium concentration was determined in quadruplicates for diets and duplicates for excreta based on the method reported by Leone et al. (1973). Briefly, 0.25 g of digesta or feed was added to threaded glass test tubes and ashed at 580°C for 10 h; 0.8 g of $NaSO_4$ was added to the ashed samples, which were diluted with 5 mL of H_2SO_4 and then heated at 130°C for 72 h; tube contents were diluted to 50 mL with distilled deionized water and held for 12 h at 25°C; 3 mL of feed samples or 1 mL of excreta samples plus 2 mL of 1.8 M H_2SO_4 were

added to glass test tubes with 150 µL of H_2O_2; and after allowing 30 min for color development, absorbance was measured on a spectrophotometer (DU 730, Beckman Coulter, Brea, CA) at 410 nm.

Excreted TiO_2 recovered at each hourly collection was expressed as a cumulative percentage of the total TiO_2 recovered during the 12 h collection for each pen. The cumulative excretion curves of each experimental diet were sigmoidal in shape. The time required to reach 1% (**T1**) and 50% (**T50**) TiO_2 excretion (Ferrando et al., 1987) was estimated from the cumulative excretion curves with non-linear regression (PROC NLIN, SAS, 2009) using a modified Weibull model (Murthy et al., 2004):

$$E_{(t)} = E_{\max} - \left(E_{\max} - E_{\min}\right)\exp\left[-C\left(\frac{t}{lt}\right)^{\beta}\right]$$

where $E_{(t)}$ is the cumulative excretion at time t in h, $E_{\max}$ is the asymptote of the cumulative TiO_2 excretion, $E_{\min}$ is the initial excreted TiO_2, *lt* is the scale parameter, and β is the shape parameter. The constant (*C*) was added to the model so that *lt* represents the time required to reach a specific proportion of excreted TiO_2 (T1, $C = \log(1/0.01)$; T50, $C = \log(1/0.5)$). Estimates of $E_{\min}$ and $E_{\max}$ were maintained at 0 and 1, respectively, to represent initial and final cumulative excretion. Estimates of $\beta \pm SE$ and root mean square error for Weibull models fitted to cumulative excretion data were 3.18 $\pm$ 0.10, 3.25 $\pm$ 0.10, 2.44 $\pm$ 0.10, and 2.79 $\pm$ 0.10 and 0.035, 0.036, 0.049, and 0.041 for CSM-MBM, CSM-DDGS, SP-MBM, and SP-DDGS, respectively. Mean retention time (**MRT**) was calculated using the following equation (Coombe and Kay, 1965):

$$\text{MRT} = \Sigma\left(x_i \times t_i\right) / \Sigma x_i$$

where x_i is the amount of TiO_2 excreted at the i^{th} collection at t_i h.

Apparent Ileal Amino Acid Digestibility Assay

Immediately after the final excreta collection in the ROP assay, experimental diets without TiO_2 were replaced with experimental diets containing TiO_2 for the remainder of the experiment. An AIAAD assay was conducted on d 22. Eight birds per pen were euthanized via CO_2 asphyxiation and digesta were collected by gently flushing the terminal ileum (4 to 30 cm proximal to the ileo-cecal junction) using deionized water. Samples were pooled and frozen for later analysis. Frozen digesta samples were prepared as aforementioned for excreta. Complete amino acid content of the diets and digesta were analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratory in quadruplicates for diets and duplicates for digesta (method 982.30 E (a,b,c), AOAC, 2006). Performic acid oxidation (method 985.28; AOAC, 2006) was conducted before acid hydrolysis for the determination of Met and Cys, whereas all other amino acids were determined after acid hydrolysis. Titanium dioxide concentration of the digesta was determined in duplicate using the method previously described for excreta. Apparent ileal amino acid digestibility (**AIAAD**) was calculated with the following equation (Lemme et al., 2004): $AIAAD = ((AA / TiO_2)_{diet} - (AA / TiO_2)_{digesta}) / (AA / TiO_2)_{diet}$.

Statistics

Data were analyzed using a randomized complete block design (SAS, 2009). Cage location was the blocking factor. Each of the 4 dietary treatments was represented by 6 replicate cages. Analysis of variance was performed using PROC MIXED (SAS, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are fixed factor level effects corresponding to the j^{th} diet type (CSM-MBM, CSM-DDGS, SP-MBM, and SP-DDGS) such that $\Sigma\tau_j = 0$; and the random error ε_{ij} are identically and independently normally distributed with mean 0 and variance σ^2. Significantly different treatment means were separated using Tukey's Honestly Significant Difference test (Tukey, 1953). Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the current study, cumulative TiO_2 excretion curves for each of the 4 experimental diets were sigmoidal in shape (Figure 3.1). Time of 50% marker excretion and MRT values for birds fed the 2 CSM diets were 4.96 and 5.60 h, respectively (Table 3.2). No significant differences ($P > 0.05$) were observed between T1, T50, or MRT in birds fed CSM-MBM or CSM-DDGS. Time of 1% TiO_2 excretion was significantly lower ($P < 0.01$) for birds fed SP-MBM than those fed CSM-MBM, CSM-DDGS or SP-DDGS, indicating a faster initial ROP. However, T50 indicated faster ($P < 0.001$) ROP for birds fed SP-DDGS than those fed CSM-DDGS or CSM-MBM, while no difference was observed ($P > 0.05$) in T50 between birds fed SP-MBM or SP-DDGS. Additionally, MRT was lower ($P < 0.001$) for birds fed SP-DDGS (5.13 h) and indicated a faster ROP than those fed CSM-DDGS, CSM-MBM, or SP-MBM, which had MRT values of 5.58, 5.62, and 5.48 h, respectively. Feed intake was greater for birds fed CSM-MBM or CSM-DDGS than those fed SP-MBM or SP-DDGS, and intake of SP-DDGS was less than SP-MBM ($P < 0.001$).

The T50 and MRT values determined in the current study were similar to those previously reported for complete diets fed to broilers (Shires et al., 1987; Hetland and Svihus, 2001). Hetland and Svihus (2001) observed a T50 value of 5.14 h for wheat-based diets fed to 15 d old broilers, while Shires et al. (1987) reported a MRT of 338 min (5.63 h) in broilers fed corn-soybean and corn-canola meal diets. However, methodology has varied among research determining ROP in poultry. In addition to diet composition and form, ROP can be affected by the strain and age of the bird (Shires et al., 1987; Washburn, 1991), fasting period (Mateos et al., 1982), ambient temperature (Wilson et al., 1980), and marker type and administration (Ferrando et al., 1987; Vergara et al., 1989; Washburn, 1991), and previous research varies substantially in these aspects. Additionally, discrepant interpretations of results can be made when considering different measurements of ROP, such as first appearance of feed or marker, the time required to reach a specified amount of marker excretion such as T1 or T50 (Ferrando et al., 1987), or the time required for a feed or marker substance to pass through a specified section of the gastrointestinal tract (MRT) (Coombe and Kay, 1965). Consequently, caution must be exercised when comparing passage time or ROP values determined in the current study with previously published literature.

In the experiment herein, an inconsistent relationship among the ROP measurements was observed, as T1 indicated faster initial ROP for SP-MBM, while T50 and MRT both indicated faster ROP for SP-DDGS. The determination of T1 resulted in analyzing low concentrations of TiO_2 in the excreta, possibly leading to increased variability. Furthermore, Ferrando et al., (1987) found no differences in T1 of birds fed chromium-mordanted wheat or bran particles varying in particle size, while differences

were observed in MRT for both particle size and ingredient source. Thus, T50 and MRT may be less variable and more accurate indicators of ROP than T1.

In the current research, experimental diets were not formulated to evaluate the influence of a single dietary component on ROP, but rather to determine if differences existed in ROP between complete diets and SP diets representative of those currently utilized in bioassays. Nevertheless, notable differences in ingredient composition may have contributed to lower T50 and MRT of SP-DDGS. A high inclusion of DDGS (76%) in SP-DDGS was required to reach 20% CP and resulted in a numerically higher fiber content compared with other experimental diets. Analyzed values for neutral detergent fiber (**NDF**) were 13.85, 13.27, 19.46, and 27.50, while values for acid detergent fiber (**ADF**) were 3.89, 4.27, 5.81, and 10.74 for CSM-MBM, CSM-DDGS, SP-MBM, and SP-DDGS, respectively. Hence, ADF content in SP-DDGS was more than 2 times that of CSM-DDGS and CSM-MBM, indicating markedly higher insoluble fiber content.

Increasing insoluble fiber content has been related to faster ROP in swine, likely due to increased digesta bulk (Ravindran et al., 1984). In poultry, the relationship between insoluble fiber and ROP is more complex due to the gizzard, which plays a prominent role in gastrointestinal motility (Duke, 1992). Gizzard activity is influenced by the source and size of insoluble dietary fiber as feed particles must be ground to a certain size before passing the gizzard (Clemens et al., 1975; Hetland et al., 2005). As a result, very coarse, insoluble fiber particles (i.e. wood shavings) may accumulate in the gizzard and result in slower ROP (Hetland et al., 2005). Conversely, large insoluble fiber may stimulate motility and ROP via increased grinding activity of the gizzard (Hetland and Svihus, 2001; Svihus et al., 2002). Hetland and Svihus (2001) determined with wheat-

based diets that a lower T50 (faster ROP) occurred when coarsely ground (1.5 mm sieve) oat hulls were included at 10% at the expense of starch and soy isolate mixture, but T50 was not reduced with the same inclusion of finely ground (0.5 mm sieve) oat hulls. Increased gizzard activity was indicated by larger gizzard weights in birds fed coarsely ground oat hulls.

Cao et al. (2003) reported a faster ROP in layers with the addition of finely powdered (70 μm mesh) cellulose to purified diets consisting of isolated soybean protein and cornstarch. Retention time was lower (faster ROP) in layers fed diets with 10% powdered cellulose than those fed 0 or 3.5% additional cellulose (Cao et al., 2003). In another study, relative gizzard weight did not indicate increased gizzard activity when powdered cellulose was added to wheat-based diets (Hetland et al., 2002). Thus, it is likely that increased ROP in poultry as a result of additional insoluble dietary fiber is not entirely attributed to increased gizzard activity. Considering the aforementioned observations of increased ROP with the addition of both coarse and fine fiber in previous studies, the lower T50 and MRT of birds fed SP-DDGS in the current study are likely a result of the higher insoluble fiber content in the diet.

In addition to fiber, supplemental fat and carbohydrate composition can influence ROP in poultry. Increasing the supplemental fat inclusion in diets fed to laying hens linearly increased time of first appearance of markers in excreta (Mateos et al., 1982). Also, first appearance of marker in excreta indicated SP diets containing soybean meal formulated with starch as the carbohydrate source had a significantly slower ROP than SP diets containing sucrose as the source of carbohydrate (Mateos and Sell, 1981). In the experiment herein, poultry oil was included at 5.00% in SP diets and 2.73 and 4.13% for

CSM-MBM and CSM-DDGS, respectively. Additionally, SP diets were formulated with purified dextrose and starch as carbohydrate sources, while corn was the primary carbohydrate source in the CSM diets. Therefore, the notable differences in these chemical components may have influenced the ROP of the experimental diets.

In the results reported herein, broilers fed either SP-MBM or CSM-MBM had similar T50 and MRT values indicating that nutrient utilization of MBM in SP or CSM diets is not influenced by differences in ROP between the 2 diet types. This observation provides further confidence in the application of amino acid digestibility values of MBM determined with SP diets. Conversely, the faster ROP in broilers fed SP-DDGS compared with those fed CSM-DDGS, as indicated by T50 and MRT, suggest nutrient utilization of DDGS may be influenced by differences in ROP between SP or CSM diets. However, this may only be true for high inclusions of DDGS such as the 76% inclusion in this experiment to reach 20% CP for SP-DDGS. The hypothesis that slower ROP results in increased nutrient utilization suggests the faster ROP of SP-DDGS observed in the current research may result in less efficient nutrient utilization of DDGS when fed in SP diets than in CSM diets. On the contrary, previous research suggests energy utilization of DDGS by broilers was greater in SP diets than in CSM diets. Adeola and Ileleji (2009) determined the AME_n of corn DDGS in broilers fed a SP diet to be 2,963 kcal/kg, approximately 180 kcal/kg higher than birds fed DDGS in a CSM diet (2,787 kcal AME_n/kg). However, AME_n was determined using the regression method with identical inclusions of DDGS (0, 30, or 60%) in the 2 diet types, and it is unknown whether a difference in ROP exists between diet types with the same inclusion of DDGS.

Rate of passage is a limiting factor for feed intake, and it is generally accepted that faster ROP allows increased feed intake. In the current study, feed intake was greater for birds fed CSM diets than those fed SP diets, and intake of SP-DDGS was less than SP-MBM. Increased feed intake of CSM diets compared with SP diets may have been due to a more desirable palatability of CSM diets. Lower feed intake of birds fed SP-DDGS than those fed SP-MBM may have been a result of the high fiber content of SP-DDGS. Although moderate amounts of dietary fiber may lead to increased feed intake in broilers, the digesta bulking effect of dietary fiber can induce satiety and cause lower feed intake of high fiber diets (Mateos et al., 2012).

Apparent ileal amino acid digestibility coefficients were higher ($P < 0.05$) for all amino acids in birds fed CSM-MBM diets than those fed SP-MBM diets except for His and Gly, which were similar for both the MBM-containing diets (Table 3.3). Broilers fed CSM-DDGS and SP-DDGS diets had similar AIAAD coefficients for Leu, otherwise AIAAD coefficients were higher ($P < 0.05$) for birds fed CSM-DDGS. Regarding the 2 SP diets, AIAAD coefficients were usually similar or higher ($P < 0.05$) in birds fed SP-DDGS, with the exception of Lys, His, and Gly, which were higher ($P < 0.05$) for birds fed SP-MBM. Apparent ileal amino acid digestibility coefficients determined for birds fed SP-MBM and SP-DDGS diets in the current study were in close agreement with previous research (Adedokun et al., 2007b; 2008) for porcine MBM and DDGS in 21 d old broilers. Direct correlation of AIAAD and ROP values determined in the current study is not possible due to the numerous factors affecting AIAAD and confounding differences in composition of the experimental diets. However, AIAAD values do serve

as a point of reference for comparing nutrient utilization of the experimental diets by the birds.

In conclusion, no differences were observed in T50 or MRT between the 2 MBM-containing diets. On the other hand, broilers fed SP-DDGS had lower T50 and MRT than those fed CSM-DDGS, thereby indicating faster ROP. Consequently, depending on ingredient type and inclusion, the ROP of SP diets may differ from that of CSM diets in broilers. However, the magnitude of which the observed differences in ROP may impact broiler growth performance has yet to be determined.

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Table 3.1 Ingredient and calculated composition of diets fed to broiler chicks for determination of rate of passage and apparent ileal amino acid digestibility

	Diet[1]			
Ingredient, % "as-fed"	CSM-MBM	CSM-DDGS	SP-MBM	SP-DDGS
Ground corn	62.41	56.69	–	–
Soybean meal (48% CP)	26.25	29.45	–	–
Dextrose	–	–	49.45	10.14
Meat and bone meal (53% CP)	5.00	–	37.50	–
Distiller's dried grains with solubles (26% CP)	–	5.00	–	76.00
Poultry oil	2.73	4.13	5.00	5.00
Solkafloc[2]	–	–	5.00	5.00
Limestone	0.66	1.08	–	2.16
Sodium chloride	0.35	0.45	–	0.20
Dicalcium phosphate	0.75	1.38	–	–
Potassium chloride	–	–	0.60	–
Magnesium oxide	–	–	0.15	–
Choline chloride	–	–	0.25	0.25
Sodium bicarbonate	–	–	0.80	–
DL-Met	0.30	0.28	–	–
L-Lys·HCl	0.19	0.19	–	–
L-Thr	0.11	0.10	–	–
Vitamin and mineral premix[3]	0.75	0.75	0.75	0.75
Titanium dioxide	0.50	0.50	0.50	0.50
Calculated Analysis, %				
CP[4]	20.2	20.2	20.1	20.1
Ca	0.80	0.80	3.24	0.95
Non-phytate P	0.40	0.40	1.76	0.30
Na	0.21	0.21	0.41	0.44
Analyzed values, % DM				
CP	22.1	21.4	20.6	19.7
Crude fat	6.6	7.6	9.3	11.7
Neutral detergent fiber	13.9	13.3	19.5	27.5
Acid detergent fiber	3.9	4.3	5.8	10.7

[1]Abbreviations: CSM = Corn-soybean meal, SP = semi-purified, MBM = meat and bone meal, DDGS = distiller's dried grains with solubles.

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

[3]Vitamin and mineral premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg, Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

[4]CP = (N × 6.25)

Table 3.2 Rate of passage, retention times, and feed intake of experimental diets fed to broiler chicks from 14 to 18 d of age[1]

Item[2]	FI[3] (g/bird)	T1[4]	T50[5]	MRT[6]
		(h)		
CSM-MBM	12.52[a]	1.33[a]	4.97[a]	5.62[a]
CSM-DDGS	12.69[a]	1.36[a]	4.94[a]	5.58[a]
SP-MBM	10.20[b]	0.87[b]	4.69[ab]	5.48[a]
SP-DDGS	8.04[c]	1.02[ab]	4.47[b]	5.13[b]
SEM	0.49	0.05	0.03	0.03
P-value	< 0.001	< 0.01	< 0.001	< 0.001

[a-b] Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]Values represent least-squares means of 6 replicate cages with 12 birds per cage at 14 d of age.

[2]CSM = corn-soybean meal, SP = semi-purified, MBM = meat and bone meal, DDGS = distiller's dried grains with solubles.

[3]FI = feed intake during 2 h access to TiO^2 containing diets.

[4]T1 = Time of 1% marker concentration in excreta.

[5]T50 = Time of 50% marker concentration in excreta.

[6]MRT = Mean retention time.

Table 3.3 Apparent ileal amino acid digestibility coefficients of corn-soybean meal or semi-purified diets containing meat and bone meal or distiller's dried grains fed to broiler chicks from 14 to 22 d of age[1]

	AIAAD coefficients[2] (%)				
Amino acid	CSM-MBM	CSM-DDGS	SP-MBM	SP-DDGS	SEM[3]
Indispensible amino acids					
Arg	89.4[a]	90.1[a]	78.2[b]	79.5[b]	0.7
Cys	79.0[ab]	79.9[a]	34.7[c]	72.7[b]	1.7
His	72.2[b]	75.3[a]	73.3[ab]	58.5[c]	0.8
Ile	84.0[a]	84.9[a]	70.3[c]	75.0[b]	0.8
Leu	84.9[a]	85.8[a]	72.5[b]	84.9[a]	0.7
Lys	89.2[a]	88.1[a]	73.9[b]	65.3[c]	0.9
Met	94.1[a]	93.7[a]	73.5[c]	82.4[b]	0.6
Phe	84.8[a]	86.0[a]	74.6[c]	81.4[b]	0.7
Thr	81.7[a]	82.1[a]	65.7[b]	67.3[b]	1.0
Trp	87.1[a]	87.3[a]	75.7[b]	75.0[b]	0.7
Val	82.4[a]	83.0[a]	70.4[b]	73.8[b]	1.2
Dispensible amino acids					
Ala	84.8[ab]	85.5[a]	78.5[c]	82.6[b]	0.7
Asp	82.5[a]	84.4[a]	63.5[c]	70.1[b]	1.0
Glu	88.2[a]	89.2[a]	71.9[c]	82.3[b]	0.8
Gly	80.8[ab]	82.5[a]	79.6[a]	70.1[b]	0.8
Pro	83.8[a]	85.4[a]	74.7[c]	79.9[b]	0.8
Ser	82.5[a]	83.7[a]	64.3[c]	76.5[b]	1.1
Tyr	84.4[ab]	86.0[a]	70.0[c]	81.6[b]	0.8

[a-b] Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]Values represent least-squares means of 6 replicate cages with 12 birds per cage at 14 d of age.

[2]AIAAD = apparent ileal amino acid digestibility, CSM = corn-soybean meal, SP = semi-purified, MBM = meat and bone meal, DDGS = distiller's dried grains with solubles.

[3]Pooled standard error.

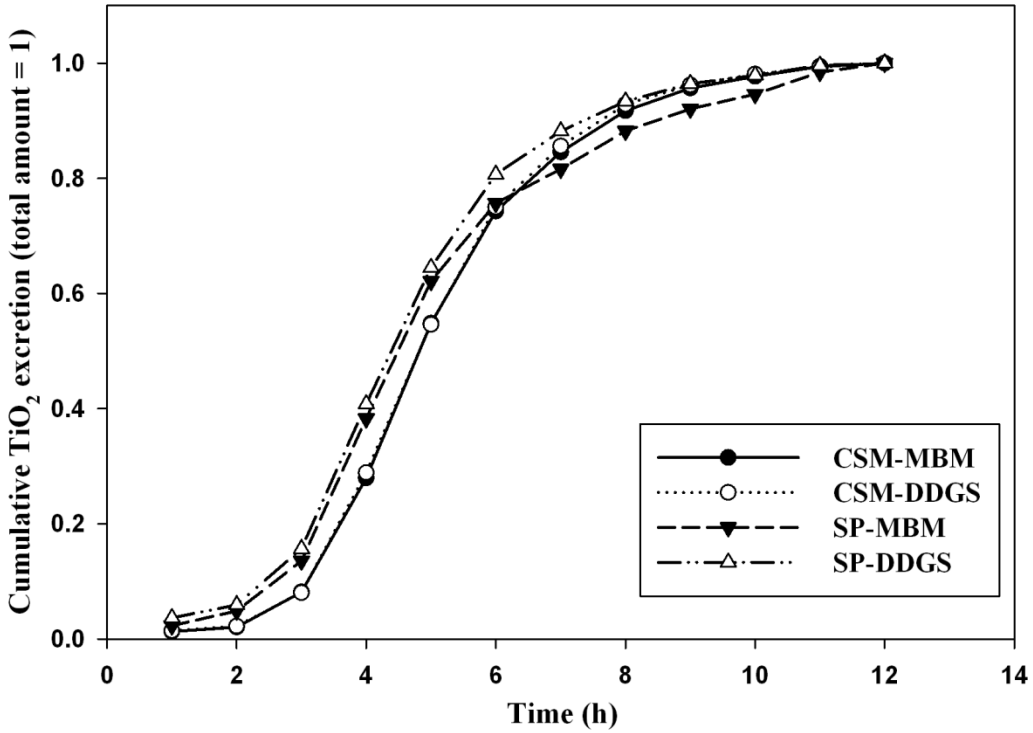

Figure 3.1 Cumulative titanium dioxide excretion curves of corn-soybean meal-based diets (CSM) and semi-purified diets (SP) containing either meat and bone (MBM) or distiller's dried grains with solubles (DDGS) fed to broiler chicks from 14 to 18 d of age. Rate of passage was determined from 6 replicate cages of birds (12 birds/cage) at 18 d of age.

IV. RELATIONSHIP BETWEEN IN VITRO ASSAYS AND STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY OF ANIMAL PROTEIN MEALS IN BROILERS

ABSTRACT

Two identical trials were conducted to determine the relationship of a novel digestive enzyme assay, Poultry Complete IDEA (PC IDEA), and the pepsin digestibility assay with standardized ileal amino acid digestibility (SIAAD) of 20 animal protein meals (APM) fed to broilers from 25 to 30 d of age. Animal protein meals included 10 meat and bone meals (MBM) consisting of bovine, porcine, or mixed bovine and porcine raw materials (BP), and 10 animal protein blends containing animal proteins from various species. Treatments consisted of 20 semi-purified diets containing 1 APM as the sole source of dietary amino acids (AA), and 1 N-free diet to determine endogenous ileal AA flow. With the exception of the N-Free diet, diets were formulated to contain 20% CP. In each trial, 756 Ross × Ross 708 male broilers were housed in battery cages and randomly assigned to 21 dietary treatments from 25 to 30 d of age (12 birds per cage; 3 replicate cages). Ileal digesta were collected on d 30 for determination of SIAAD. Pepsin digestibility and PC IDEA were determined for APM samples from each experimental diet (3 replicates per trial; 6 total replicates). Pepsin digestibility and PC IDEA were both correlated ($P < 0.001$) with SIAAD for each AA. Multiple linear regression of PC IDEA and pepsin digestibility on SIAAD resulted in the following equations: % Lys SIAAD = [–9.65 + (0.38 × % PC IDEA predicted Lys digestibility) + (0.69 × % pepsin digestibility)], % Met SIAAD = [–35.95 + (0.62 × % PC IDEA

predicted Met digestibility) + (0.75 × % pepsin digestibility)], % Thr SIAAD = [–77.5 + (0.39 × % PC IDEA predicted Thr digestibility) + (1.37 × % pepsin digestibility)]. Values of R^2 were 0.46, 0.47, and 0.55 for Lys, Met, and Thr, respectively. The relatively low R^2 values may have been due to the limited range in SIAAD observed for the 20 APM, and additional data on APM varying in SIAAD are needed.

INTRODUCTION

Animal protein meals (**APM**) are excellent sources of amino acids (**AA**), energy, and minerals. In the past, poultry by-product meal (**PBM**) was utilized as the primary APM in poultry diets due to its availability and perceived low nutrient variability. However, a substantial portion of PBM is now marketed to the pet-food industry at a premium price. Pet food-grade PBM contains highly digestible parts and is higher in AA concentrations than feed-grade PBM, which is more variable due to higher proportions of low quality by-products such as feathers, heads, and feet (Dozier et al., 2003). Consequently, poultry nutritionists are utilizing more meat and bone meal (**MBM**) and protein blends in broiler diets. Meat and bone meal is comprised of inedible products from slaughtering facilities that can originate from single or mixed species, as well as trimmings and waste from butcher shops, grocery stores, and restaurants. The various raw materials and processing conditions used by renderers in producing MBM yields products that are highly variable in chemical composition and protein quality (Johnston and Coon; 1979, Parsons et al., 1997; Wang and Parsons, 1998a; Ravindran et al., 2002). In an effort to reduce nutrient variability, animal protein blends are formulated to desired nutrient specifications by combining multiple animal protein sources with supplemental amino acids.

Poultry nutritionists may limit the inclusion of APM or utilize safety margins for AA to reduce the likelihood of impaired bird performance due to variability in AA composition. Formulating broiler diets containing APM based on digestible AA content rather than total AA content of APM allows a better prediction of dietary AA quality and growth performance, thereby reducing the need for wide safety margins (Rostagno et al., 1995; Wang and Parsons, 1998b). Bioassays are often employed to determine AA digestibility of APM for poultry. The standardized ileal AA digestibility (**SIAAD**) utilizes growing broilers, enables *ad libitum* feeding, and accounts for age-appropriate basal endogenous losses (Lemme et al., 2004). There is a growing consensus that the SIAAD assay is a suitable method for evaluating the AA digestibility of feed ingredients (Bryden and Li, 2010). However, bioassays are costly and require a significant investment of time and labor. Therefore, rapid and accurate *in vitro* assays that predict AA digestibility would be useful tools for broiler nutritionists to evaluate currently available APM.

The pepsin digestibility assay is rapid and relatively inexpensive, and has been widely used for evaluating protein quality of feed ingredients for many years (Parsons et al., 1997). Correlations of the pepsin digestibility assay with *in vivo* digestibility measurements for APM in poultry have been evaluated with inconsistent results (Parsons et al., 1997; Ravindran et al., 2002). More recently, a novel digestive enzyme assay (Poultry Complete IDEA, Novus International, Inc., St. Charles, MO) (**PC IDEA**) has been developed for predicting AA digestibility of MBM, PBM, feather meal, and other meat meals (Schasteen et al., 2002). A strong relationship between a similar assay (SBM IDEA, Novus International, Inc., St. Charles, MO) and AA digestibility in precision-fed

roosters for soybean meal has been reported, with R^2 values of 0.86, 0.88, 0.88, 0.86, and 0.90 for Lys, Met, Thr, Val, and Ile, respectively. However, published literature on the relationship between PC IDEA and AA digestibility of commercially available APM is lacking. Thus, correlations of PC IDEA and pepsin digestibility with SIAAD of commercially available APM in broilers are of interest to poultry nutritionists.

The objective of this study was to evaluate PC IDEA and pepsin digestibility as indicators of SIAAD of 20 APM varying in chemical composition fed to broilers from 25 to 30 d of age. Correlations of PC IDEA and pepsin digestibility were assessed. Furthermore, multiple linear regression was used to determine the degree of variation in SIAAD that could be explained by these 2 *in vitro* assays.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the experimental protocol involving live birds (PRN 2009-1675).

Animal Protein Meals

Twenty-nine APM were obtained from renderers, blenders, and poultry feed mills throughout the United States. These APM were analyzed at Auburn University for moisture (method 934.01; AOAC, 2006) and CP (method 968.06; AOAC, 2006), with CP being calculated by multiplying percent N (Rapid N Cube, Elementar Analysensyteme GmbH, Hanau, Germany) by a correction factor of 6.25. Crude fat [(method 920.39 (a)], ash (method 942.05) and complete mineral profile (method 968.08) were determined by the University of Missouri Agricultural Experiment Station Chemical Laboratory according to procedures of the AOAC, 2006. Twenty APM were chosen that encompassed a wide range in nutrient composition (Table 4.1). These included 10 MBM

consisting of bovine (3 samples), porcine (3 samples), or mixed bovine and porcine (4 samples) raw materials (**BP**), and 10 blended APM containing various animal by-products and additional ingredients such as plant-based proteins and supplemental amino acids. Each of the 20 APM sources were divided into 6 aliquots (3 per trial) that were used for experimental diets so independent PC IDEA, pepsin digestibility, and SIAAD values could be obtained for each replicate cage.

Dietary Treatments

Twenty-one dietary treatments consisted of 20 semi-purified diets containing a single APM as the only source of AA, and 1 N-free diet for determination of basal endogenous AA losses. Experimental diets containing APM were formulated to contain 20% CP (Table 4.2). All diets were formulated to be adequate in vitamins and minerals (NRC, 1994) and maintain a dietary electrolyte balance (**DEB**) between 140 and 220 milliequivalent (**mEq**). Titanium dioxide was used (0.5% inclusion) as an inert marker for all diets. Diets were mixed (126 diets) for each cage, with each of the 21 dietary treatments being represented by 6 replications (3 replicates per trial). All dietary treatments were provided in mash form.

Bird Husbandry

In each trial, 756 Ross × Ross 708 (Aviagen, Inc., Huntsville, AL) male chicks were obtained from a commercial hatchery and received vaccinations for Marek's disease, Newcastle disease, and infectious bronchitis. Chicks were placed into 63 grower battery cages (Petersime**,** Gettysburg, OH) (12 birds per cage; 0.04 m^2/bird), and each cage was equipped with 1 trough feeder and 1 trough waterer. Battery cages were located in a solid-sided house with temperature control. Temperature was set to 33^{o}C at

placement and was decreased gradually to 24°C by the end of experimentation. A 23L:1D lighting schedule was used until d 25. On d 25, a 12L:12D lighting schedule was implemented to obtain sufficient feed intake approximately 3 h prior to collection to ensure adequate amounts of ileal digesta could be collected on d 30. Broilers were fed a common corn-soybean meal starter diet (AME_n, 3,075 kcal/kg; digestible Lys, 1.22%; digestible TSAA, 0.92%; digestible Thr, 0.83%; Ca, 0.90%; and non-phytate P, 0.45%) that met or exceeded NRC (1994) nutrient recommendations from 1 to d 24 of age.

Standardized Ileal Amino Acid Digestibility Assay

On d 25, chicks were randomly assigned to 1 of 21 experimental diets. Following a 5-d acclimation period, ileal digesta were collected for the determination of SIAAD on d 30. Eight birds per pen were euthanized via CO_2 asphyxiation and digesta were collected by gently flushing the terminal ileum (4 to 30 cm proximal to the ileo-cecal junction) using deionized water. Samples were pooled and frozen (-20ºC) for later analysis. Frozen digesta samples were thawed, lyophilized, and ground using an electric coffee grinder to provide a finely ground sample while avoiding significant loss. Complete AA content of the diets and ileal digesta were analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratory in duplicates for diets and digesta [method 982.30 E (a,b,c), AOAC, 2006]. Performic acid oxidation (method 985.28; AOAC, 2006) was conducted before acid hydrolysis for the determination of Met and Cys, whereas all other amino acids were determined after acid hydrolysis.

Titanium concentration of diets and digesta were determined in duplicate based on the method reported by Leone et al. (1973). Briefly, 0.25 g of digesta or feed was added to threaded glass test tubes and ashed at 580°C for 10 h; 0.8 g of $NaSO_4$ was added

to the ashed samples, which were diluted with 5 mL of H_2SO_4 and then heated at 130°C for 72 h; tube contents were diluted to 50 mL with distilled deionized water and held for 12 h at 25°C; 3 mL of feed samples or 1 mL of excreta samples plus 2 mL of 1.8 M H_2SO_4 were added to glass test tubes with 150 µL of H_2O_2; and after allowing 30 min for color development, absorbance was measured on a spectrophotometer (DU 730, Beckman Coulter, Brea, CA) at 410 nm.

Apparent ileal amino acid digestibility (**AIAAD**) was calculated with the following equation (Lemme et al., 2004): $AIAAD = ((AA/TiO_2)_{diet} - (AA/TiO_2)_{digesta})/(AA/TiO_2)_{diet}$. Ileal endogenous AA (**IEAA**) flow in broilers fed the N-free diet was calculated as milligrams of AA flow per kg of dry matter intake (**DMI**) using the flowing equation (Moughan et al., 1992; Adedokun et al., 2007): IEAA, mg/kg of DMI = Ileal AA, mg/kg × $[(TiO_2)_{diet}/(TiO_2)_{digesta}]$. Apparent IAAD coefficients were standardized using the determined IEAA flows with the following equation (Adedokun et al., 2007): SIAAD = AIAAD + [(IEAA flow g/kg of DMI)/(AA content of the diet, g/kg of DM)] × 100.

In Vitro Assays

In vitro assays were performed on APM samples (120 samples) from each experimental diet for a total of 6 subsamples for each of the 20 APM sources. Subsamples were finely ground with a cyclone mill (Cyclotec model number 1093, Foss North America, Inc., Eden Prairie, MN) equipped with a 1 mm screen, and any material that adhered to the mill or screen was added back to the ground sample and homogenized. Pepsin digestibility (method 971.09, AOAC, 2006) of each APM sample was determined in duplicate. For PC IDEA, ground APM samples (~400 mg) were solubilized in a

solution containing 50 mM KH_2PO_4, 0.1% NaN_3, and 50 mM EDTA adjusted to pH 6.2. A 1.0 mL sample of the solubilized APM was retained for an initial measurement, and duplicate samples (2.5 mL) were transferred to an enzyme kit for digestion (Novus International, 2012). Digestion was carried out on an end-to-end rotator for 2 h in a 37ºC incubator. Digestion was quantified by the reaction of α-amino groups with o-phthaldialdehyde (**OPA**) for initial and digested samples (Schasteen et al., 2007). The OPA reagent contained 50 mL of 0.1 *M* sodium borate, 80 mg of OPA dissolved in 2 mL of 95% ethanol, 200 µL of 2-mercaptoethanol, and 5 mL of 20% sodium dodecyl sulfate, diluted to 100 mL with water (Schasteen et al., 2007). Triplicate aliquots (10 µL) of 1 initial and 2 digested samples were combined with 1 mL of OPA reagent in disposable cuvettes, briefly inverted, and allowed to incubate for 2 min. Absorbance was measured on a spectrophotometer (DU 730, Beckman Coulter, Brea, CA) at 340 nm. The PC IDEA values were calculated using the following equation (Schasteen et al., 2007):

$$\text{PC IDEA value} = [A_{340}\,(\text{final}) - A_{340}\,(\text{initial})]/\text{percent CP},$$

where A_{340} (final) is the average absorbance of the 2 digested aliquots, A_{340} (initial) is the absorbance of the undigested solubilized sample, and percent CP of the APM sample is the CP content determined with a nitrogen analyzer (method 968.06; AOAC, 2006) (N × 6.25) (Rapid N cube, Elementar Analysensysteme Gmbh, Hanau, Germany). A control APM of known digestibility was included in each run of the assay to determine a standardization factor, which was calculated as described by Boucher et al. (2009):

$$\text{Standardization factor} = \text{Novus predetermined PC IDEA value for standard/ measured PC IDEA value for standard.}$$

The PC IDEA values were then adjusted with the standardization factor:

Corrected PC IDEA value = PC IDEA value × standardization factor.

The corrected PC IDEA values were used to calculate predicted AA digestibilities estimated from equations supplied in the kit, which are based upon regression analysis of PC IDEA values and standardized AA digestibility values determined with the precision-fed cecectomized rooster assay.

Statistics

Data were analyzed using a randomized complete block design (SAS, 2009). Pen location was the blocking factor. Each treatment was represented by 6 replications (3 replicates per trial) over time. Pearson correlations between PC IDEA, pepsin digestibility, and SIAAD were conducted using PROC CORR (SAS, 2009). Multiple linear regression (stepwise selection) of PC IDEA and pepsin digestibility as predictors of SIAAD was conducted using PROC REG (SAS, 2009) with the following model:

$$y_i = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \varepsilon_i$$

where y_i = SIAAD, β_o = intercept of the regression equation, β_j = regression coefficient, x_1 = PC IDEA predicted digestibility, x_2 = pepsin digestibility, and ε_i = random error of the regression model. The coefficient of determination (R^2), MSE of the regression equation, and the Mallows statistic [C(p)] were used to define the equation with the best fit. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

A wide range in nutrient composition of test ingredients allows for the development of robust correlations and regression equations relating *in vitro* assays with *in vivo* nutrient utilization. Twenty commercially available APM products, consisting of 10 BP-MBM and 10 animal protein blends, were obtained from commercial renderers,

blenders, and feed mills. Accordingly, nutrient composition of the APM samples was diverse (Table 4.1). Crude protein content ranged from 41.7 to 61.8%, CF from 4.1 to 13.1%, ash from 16.8 to 45.0%, Ca from 5.9 to 19.9%, P from 2.3 to 9.4%, Na from 0.34 to 1.30%, Cl from 0.10 to 1.55%, and K from 0.13 to 0.63%. The range in CP, CF, and ash contents of APM in the current study was higher than that observed by Olukosi and Adeola (2009) for 21 MBM samples, but lower than that observed by Ravindran et al. (2002) for 19 MBM produced in New Zealand. Average contents of CP (53.6%), CF (9.3%), and ash (26.7%) for APM in this experiment were in good agreement with those reported by others (Parsons et al., 1997; Ravindran et al., 2002; Adedokun and Adeola, 2005; Olukosi and Adeola, 2009).

In order to formulate semi-purified diets to 20% CP, dietary inclusions of APM ranged from 32.37 to 47.92% (Table 4.2). Dietary electrolyte balance ($Na^+ + K^+ - Cl^-$) of the APM-containing diets ranged from 145 to 214 mEq, with most diets being near the average DEB of 175 mEq. The high Na^+, K^+, and Cl^- contents of APM resulted in dietary concentrations of these elements much higher than their requirement. Therefore, an effort was made to balance the DEB among the diets with minimal Na^+, K^+, and Cl^- supplementation. The DEB of the N-free diet (172 mEq) was formulated to be similar to the average DEB of the APM-containing diets (175 mEq). Adedokun et al. (2011) reported a DEB of 108 mEq may be preferred for N-free diets because the Na^+, K^+, and Cl^- requirements can be met at this level, and that feeding N-free diets with a higher DEB may result in watery excreta. In the experiment herein, no excessively watery excreta were observed for birds fed the experimental diets.

In the current study, AA composition of the diets cannot be directly compared because inclusion levels of APM varied among diets (Table 4.3). However, AA composition of the APM sources can be calculated by dividing diet AA composition by the inclusion level of the corresponding APM source. This conversion enables direct comparison of AA composition among APM sources. As expected, AA composition of the APM sources varied substantially. Animal protein meals were most deficient in Trp, Met, Cys, and His, which averaged 0.39, 0.72, 0.82, and 0.99%, respectively. Average composition of Gly (6.55%), Glu (6.30%), Pro (4.45%), Asp (3.94%), and Ala (3.76%) indicated that these APM sources were relatively high in non-essential AA. Lysine, Thr, Val, and Ile contents averaged 2.67, 1.83, 2.61, and 1.73%, respectively. The total AA content (23 AA) of the APM ranged from 41.19 to 60.40% with an average of 52.44% and these data are in close agreement to the range (46.40 to 60.30%) and average (55.00%) total AA content in 21 MBM samples reported by Olukosi and Adeola (2009).

In addition to AA composition, the 20 APM sources varied substantially in SIAAD (Table 4.4). Standardized ileal digestibility of Lys ranged from 44.5 to 84.2% with an average of 74.8%, Met ranged from 41.4 to 85.5% with an average of 75.2%, Cys ranged from 32.0 to 76.2% with an average of 56.5%, Thr ranged from 42.1 to 81.5% with an average of 71.4%, Val ranged from 45.1 to 83.6% with an average of 74.0%, and Ile ranged from 43.8 to 84.7% with an average of 75.5%. Apparent ileal Trp digestibility could not be standardized because the low concentrations of Trp (< 0.04%) in the digesta of birds fed the N-free diet prevented accurate determination of basal endogenous Trp losses. Apparent ileal digestibility of Trp ranged from 35.5 to 69.9% with an average of 56.1%. Standardized IAAD of BP-MBM (APM 1 to 10) in this study was similar to that

reported by Adedokun et al. (2007) for Lys (73.8%), Met (72.5%), Thr (67.2%), and Val (72.5%) for 4 sources of MBM in 21 d old birds using a N-free diet. To our knowledge, no published SIAAD values are available for a range of sources similar to the animal protein blends (APM 11 to 20) in the current study.

Pepsin digestibility of APM in the current study ranged from 81.1 to 95.9%, with an average of 89.0% (Table 4.5). This is in agreement with pepsin digestibility values using 0.2% pepsin concentration for various MBM reported by Ravindran et al. (2002) (89.7%) and Hendriks et al. (2002) (89.9%), while slightly higher than 86.2% reported by Parsons et al. (1997) for 13 MBM. In the current study, Pearson correlation coefficients of pepsin digestibility with SIAAD were statistically significant ($P < 0.001$) for all AA, with an average correlation coefficient of 0.608 when all 20 APM were considered (Table 4.6). Correlation coefficients for Lys, Met, Cys, and Ile were 0.582, 0.591, 0.644, and 0.660, respectively. The strongest correlation coefficients were observed for Thr (0.714), Val (0.699), and Leu (0.698), while the weakest were for Gly (0.372), Pro (0.481), and Arg (0.491). When only BP-MBM (APM 1 to 10) or animal protein blends (APM 11 to 20) were considered, correlations coefficients for all AA were weaker and resulted in values of 0.415 and 0.463 for Lys, 0.470 and 0.400 for Met, 0.474 and 0.615 for Thr, 0.442 and 0.655 for Val, and 0.474 or 0.615 for Ile, respectively. For BP-MBM, negative correlation coefficients were significant for Gly ($r = -0.272$, $P < 0.05$), while SIAAD values of Arg, Phe, Ala, Glu, and Pro were not correlated ($P > 0.05$) with pepsin digestibility. For the animal protein blends, pepsin digestibility was not significantly ($P > 0.05$) correlated with SIAAD values of Arg, Cys, Gly, or Pro.

The AOAC (2006) recommended pepsin concentration of 0.2% was used in this study. The results of this experiment are in contrast with previous reports on the relationship of AA digestibility of APM with the pepsin digestibility assay when using a 0.2% pepsin concentration. Johnston and Coon (1979) and Parsons et al. (1997) suggested that the pepsin digestibility assay using a pepsin concentration of 0.2% may digest protein to an extent that prevents detection of subtle differences in the protein quality of MBM. These researchers reported that lowering the pepsin concentration to 0.002% improved the sensitivity of the assay. Parsons et al. (1997) determined that correlation coefficients of 0.002% pepsin digestibility with Lys digestibility in conventional and cecectomized roosters were 0.62 and 0.69, respectively. With the exception of Gly, Ravindran et al. (2002) did not detect any significant correlations between pepsin digestibility (0.2% pepsin) and apparent ileal amino acid digestibility of 19 MBM in 5 wk old broilers. Animal protein meals in previous studies were primarily limited to MBM and did not contain animal protein blends similar to APM 11 to 20 in the current study. The range of pepsin digestibility for the 20 APM sources in the current study (81.1 to 95.9%) was greater than observed by Parsons et al. (1997) (83.2 to 89.3%) and Ravindran et al. (2002) (84.3 to 94.4%). Additionally, Parsons et al. (1997) reported correlations of pepsin digestibility with AA digestibility determined in precision-fed roosters, while Ravindran et al. (2002) reported correlations with apparent rather than standardized ileal AA digestibility. The discrepancy between previous results on the relationship of 0.2% pepsin digestibility and results in this study may have been partially attributed to differences between types of APM and *in vivo* AA digestibility methods and a greater range in pepsin digestibility in the current study.

Amino acid digestibility coefficients predicted by PC IDEA were correlated ($P < 0.001$) with SIAAD for all AA when based on all 20 APM, with an average correlation coefficient of 0.513 (Table 4.6). The strongest correlation coefficients were observed for Met (0.638), Tyr (0.637), and Lys (0.630), while the weakest were for Pro (0.305), Gly (0.309), and Arg (0.329). Correlation coefficients for Cys, Ile, and Val were and 0.526, 0.595, and 0.541, respectively. When only BP-MBM (APM 1 to 10) or animal protein blends (APM 11 to 20) were considered, correlations coefficients were lower with values of 0.506 and 0.566 for Lys, 0.511 and 0.557 for Met, 0.512 and 0.421 for Thr, 0.477 and 0.394 for Val, and 0.531 or 0.508 for Ile, respectively. For BP-MBM, correlation coefficients were not significant ($P > 0.05$) for Arg, Phe, Ala, Gly, and Pro. For animal protein blends, SIAAD of Arg, Cys, Gly, and Pro were not correlated ($P > 0.05$) with PC IDEA. Generally, PC IDEA predicted digestibility coefficients were lower than observed SIAAD coefficients for essential AA, with the exception of Arg, and higher for nonessential AA, with the exception of Asp. The predicted PC IDEA AA digestibility coefficients were calculated using equations supplied with the enzyme kit, which are based upon correlations with AA digestibility determined in precision-fed roosters. Boucher et al. (2009) determined a strong relationship between IDEA predicted digestibility and digestibility determined in precision-fed roosters for fish meal (n = 5), with R^2 values of 0.73, 0.92, and 0.91 for Lys, Met, and Thr, respectively. In evaluating 3 APM, Kim et al. (2011) determined that AA digestibility was higher for 1 MBM and lower for a second MBM and PBM with the precision-fed rooster assay than with the SIAAD assay. Therefore, differences in AA digestibility of APM between the precision-

fed rooster assay and SIAAD assay are inconsistent and may have influenced correlations of PC IDEA with SIAAD in the current study.

Multiple linear regression was employed to determine the extent of variability that could be explained when using both the pepsin digestibility assay and PC IDEA as indicators of SIAAD for APM (Table 4.7). Values of R^2 for the multiple linear regression equations ranged from 0.24 to 0.55. The SIAAD of Lys was predicted with the following equation: % Lys SIAAD = [–9.65 + (0.38 × % PC IDEA predicted Lys digestibility) + (0.69 × % pepsin digestibility)], (R^2 = 0.46, MSE = 44.80). Methionine SIAAD was predicted by: % Met SIAAD = [–35.95 + (0.62 × % PC IDEA predicted Met digestibility) + (0.75 × % pepsin digestibility)], (R^2 = 0.47, MSE = 45.45). The prediction equation for Thr was: % Thr SIAAD = [–77.55 + (0.39 × % PC IDEA predicted Thr digestibility) + (1.37 × % pepsin digestibility)], (R^2 = 0.55, MSE = 42.75). Regression equations for Val and Ile resulted in R^2 values of 0.51 and 0.49, respectively. Stepwise selection did not include PC IDEA as a significant predictor of SID for Ala, Arg, Gly, Pro, Ser or Phe. For all other AA, both PC IDEA and the pepsin digestibility assay were considered significant predictors of SIAAD.

A number of factors may have attributed to the relatively low R^2 values for the prediction of SIAAD in the current study. The 20 APM were selected to provide a wide range in nutrient composition, but the range in observed SIAAD of the APM was limited. With the exception of APM-11 (44.5%), SIAAD of Lys ranged between approximately 65 and 85% with 11 of the 20 APM sources falling between 70 and 80%. There were no sources of APM that resulted in SIAAD of Lys less than 45% or between 45 to 65%. If

there had been a wider and more uniform distribution of SIAAD, stronger correlations and more robust prediction equations may have been observed.

In conclusion, these data indicated a significant relationship of PC IDEA and pepsin digestibility with SIAAD for APM diverse in AA quality, and resulted in the following prediction equations for Lys, Met, and Thr: % Lys SIAAD = [–9.65 + (0.38 × % PC IDEA predicted Lys digestibility) + (0.69 × % pepsin digestibility)], % Met SIAAD = [–35.95 + (0.62 × % PC IDEA predicted Met digestibility) + (0.75 × % pepsin digestibility)], % Thr SIAAD = [–77.5 + (0.39 × % PC IDEA predicted Thr digestibility) + (1.37 × % pepsin digestibility)]. Values of R^2 for prediction equations for SIAAD of Lys, Met, Cys, Thr, Val, and Ile were 0.46, 0.47, 0.44, 0.55, 0.51, and 0.49, respectively. The relatively low R^2 values may have been due to the limited range in SIAAD observed for the 20 APM, and additional data on APM varying in SIAAD are needed.

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Table 4.1 Nutrient composition of animal protein meals for determination of standardized ileal amino acid digestibility from 25 to 30 d of age and correlation of *in vitro* indicators of amino acid digestibility[1]

			%, "as-is"								
Sample[2]	Type[3]	Source	Moisture	CP	Crude Fat	Ash	Ca	P	Na	K	Cl
APM-1	MBM – porcine	Packer	3.8	54.1	10.9	25.5	8.6	4.7	0.53	0.47	0.31
APM-2	MBM – mixed	Renderer	4.0	47.5	10.0	32.7	10.8	5.4	0.97	0.37	0.90
APM-3	MBM – mixed	Renderer	3.6	51.4	10.9	27.1	9.1	4.3	1.25	0.48	1.37
APM-4	MBM – mixed	Renderer	2.8	55.6	9.8	19.2	9.0	4.7	0.83	0.46	0.59
APM-5	MBM – bovine	Packer	3.7	50.9	7.9	33.5	11.8	6.1	0.66	0.32	0.34
APM-6	MBM – porcine	Packer	6.2	50.7	10.4	27.6	10.1	5.4	0.50	0.38	0.25
APM-7	MBM – mixed	Renderer	3.0	48.9	10.1	32.5	11.3	5.9	1.06	0.39	0.99
APM-8	MBM – porcine	Packer	2.7	53.0	10.4	28.0	10.0	5.3	0.56	0.44	0.30
APM-9	MBM – bovine	Packer	3.2	44.3	9.2	38.2	15.2	7.4	0.72	0.27	0.35
APM-10	MBM – bovine	Packer	6.3	41.7	4.1	45.0	19.9	9.4	0.55	0.13	0.10
APM-11	Animal protein blend	Renderer	1.2	55.8	13.1	24.4	7.8	4.1	0.85	0.63	0.84
APM-12	Animal protein blend	Renderer	5.0	61.5	9.2	19.9	7.3	3.1	0.41	0.28	0.33
APM-13	Animal protein blend	Renderer	4.1	60.9	9.7	24.6	8.7	2.3	0.47	0.31	0.36
APM-14	Animal protein blend	Renderer	6.0	50.1	6.5	16.8	5.9	3.5	0.34	0.62	0.23
APM-15	Animal protein blend	Renderer	5.2	58.6	9.2	20.1	7.5	3.6	0.44	0.22	0.52
APM-16	Animal protein blend	Renderer	3.9	56.2	10.4	24.5	8.6	4.3	0.81	0.41	0.74
APM-17	Animal protein blend	Renderer	4.0	57.3	9.6	19.3	6.0	3.2	0.81	0.61	0.97
APM-18	Animal protein blend	Renderer	4.5	61.8	10.5	19.4	6.7	3.2	0.66	0.36	0.63
APM-19	Animal protein blend	Renderer	5.7	56.2	5.0	28.1	10.0	5.1	0.47	0.21	0.37
APM-20	Animal protein blend	Renderer	4.2	56.1	8.6	28.4	8.5	4.4	1.30	0.35	1.55

[1]All values reported as percentage on an as-is basis.

[2]APM = animal protein meal.

[3]MBM = meat and bone meal. Mixed MBM contain both bovine and porcine raw materials. Animal protein blends contain various animal by-products and may contain other ingredients such as grocery trimmings, plant-based proteins, or supplemental amino acids.

Table 4.2 Ingredient composition of diets fed to broilers from 25 to 30 d of age for determination of standardized ileal amino acid digestibility[1]

Ingredient, % "as-fed"	Diet										
	N-free	APM-1	APM-2	APM-3	APM-4	APM-5	APM-6	APM-7	APM-8	APM-9	APM-10
Animal protein meal	–	36.95	42.14	38.91	35.97	39.29	39.46	40.87	37.71	45.11	47.92
Dextrose	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53
Starch	45.09	12.15	6.83	10.14	13.03	9.63	9.42	8.13	11.26	3.79	0.89
Solkafloc[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Poultry oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium sulfate	0.80	0.50	0.85	0.77	0.82	0.83	0.68	0.82	0.69	0.92	0.76
Potassium chloride	0.34	0.10	–	–	–	0.07	0.14	–	0.11	–	0.25
Sodium bicarbonate	0.84	0.12	–	–	–	–	0.12	–	0.05	–	–
Deflourinated phosphate	1.85	–	–	–	–	–	–	–	–	–	–
Calcium carbonate	0.90	–	–	–	–	–	–	–	–	–	–
Magnesium oxide	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin and mineral premix[3]	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis[4]											
CP, %	–	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
DEB, mEq	172	171	190	179	188	184	171	191	170	214	185
Analyzed CP, % DM	–	20.3	20.5	21.1	20.5	20.7	21.2	20.7	21.0	21.9	20.3

[1]All values reported as percentage on an as-is basis unless otherwise noted. Animal protein meals (APM) as described Table 4.1.

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

[3]Vitamin and mineral premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg; Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

[4]CP (N × 6.25); DEB = dietary electrolyte balance; mEq = milliequivalent values of $Na^+ + K^+ - Cl^-$.

Table 4.2 (continued)

Ingredient, % "as-fed"	Diet									
	APM-11	APM-12	APM-13	APM-14	APM-15	APM-16	APM-17	APM-18	APM-19	APM-20
Animal protein meal	35.84	32.51	32.86	39.90	34.14	35.57	34.92	32.37	35.57	35.64
Dextrose	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53	38.53
Starch	13.32	16.00	15.76	9.00	14.35	13.39	14.20	16.46	12.99	13.26
Solkafloc[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Poultry oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium sulfate	0.66	0.84	0.84	0.42	1.03	0.86	0.70	0.94	0.96	0.92
Potassium chloride	–	0.12	0.10	0.16	–	–	–	–	0.06	–
Sodium bicarbonate	–	0.35	0.26	0.34	0.30	–	–	0.05	0.24	–
Deflourinated phosphate	–	–	–	–	–	–	–	–	–	–
Calcium carbonate	–	–	–	–	–	–	–	–	–	–
Magnesium oxide	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin and mineral premix[3]	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated Analysis[4]										
CP, %	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
DEB, mEq	164	169	168	169	168	168	145	159	173	164
Analyzed CP, % DM	20.3	20.5	19.8	21.0	21.2	19.8	20.4	20.5	21.3	19.9

[1]All values reported as percentage on a DM basis unless otherwise noted. Animal protein meals (APM) as described in Table 4.1.

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

[3]Vitamin and mineral premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg; Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

[4]CP (N × 6.25); DEB = dietary electrolyte balance; mEq = milliequivalent values of $Na^+ + K^+ - Cl^-$.

Table 4.3 Amino acid content of semi-purified diets containing animal protein meals fed to broilers from 25 to 30 d of age[1]

	Indispensible amino acid content (%)										
Diet[2]	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
APM-1	1.40	0.20	0.57	0.60	1.28	0.83	0.30	0.68	0.66	0.16	0.88
APM-2	1.35	0.18	0.33	0.56	1.18	0.95	0.25	0.65	0.62	0.14	0.84
APM-3	1.32	0.22	0.39	0.65	1.30	1.02	0.27	0.71	0.65	0.15	0.94
APM-4	1.33	0.18	0.45	0.63	1.37	1.14	0.32	0.74	0.68	0.17	0.96
APM-5	1.42	0.16	0.31	0.53	1.15	1.00	0.26	0.62	0.60	0.14	0.80
APM-6	1.43	0.20	0.37	0.58	1.24	1.14	0.30	0.67	0.65	0.15	0.86
APM-7	1.38	0.16	0.36	0.56	1.22	1.01	0.26	0.67	0.62	0.14	0.86
APM-8	1.42	0.17	0.37	0.56	1.20	1.06	0.29	0.66	0.62	0.15	0.84
APM-9	1.45	0.15	0.30	0.51	1.09	0.98	0.25	0.61	0.57	0.12	0.78
APM-10	1.52	0.09	0.28	0.45	0.98	0.93	0.25	0.57	0.52	0.11	0.72
APM-11	1.27	0.12	0.37	0.66	1.22	1.03	0.34	0.67	0.61	0.12	0.83
APM-12	1.31	0.59	0.34	0.79	1.48	1.00	0.27	0.83	0.78	0.14	1.16
APM-13	1.29	0.55	0.37	0.82	1.52	0.98	0.28	0.84	0.80	0.14	1.16
APM-14	1.49	0.40	0.35	0.71	1.42	0.86	0.26	0.85	0.74	0.16	1.02
APM-15	1.30	0.56	0.41	0.72	1.60	1.09	0.23	0.92	0.78	0.16	1.23
APM-16	1.26	0.35	0.33	0.65	1.29	0.83	0.24	0.72	0.69	0.15	0.96
APM-17	1.33	0.35	0.36	0.73	1.37	1.01	0.27	0.78	0.71	0.17	1.04
APM-18	1.33	0.50	0.31	0.78	1.45	0.98	0.24	0.81	0.77	0.15	1.16
APM-19	1.39	0.42	0.44	0.60	1.57	1.11	0.23	0.91	0.75	0.15	1.20
APM-20	1.37	0.32	0.32	0.65	1.29	0.88	0.24	0.72	0.68	0.14	0.97
N-free	0.01	0.00	0.00	0.01	0.02	0.01	0.00	0.01	0.01	< 0.04	0.01

[1]All values reported as percentage on a DM basis. Samples were analyzed in duplicate.
[2]Animal protein meals (APM) as described in Table 4.1.

Table 4.3 (continued)

Diet[2]	Dispensible amino acid content (%)							
	Ala	Asp	Glu	Gly	Pro	Ser	Tyr	Total[3]
APM-1	1.44	1.52	2.47	2.51	1.56	0.70	0.46	19.46
APM-2	1.49	1.41	2.28	2.77	1.70	0.72	0.39	19.47
APM-3	1.45	1.48	2.45	2.45	1.61	0.76	0.47	19.27
APM-4	1.50	1.54	2.41	2.44	1.53	0.71	0.48	19.66
APM-5	1.53	1.39	2.29	2.94	1.76	0.68	0.39	19.30
APM-6	1.50	1.52	2.49	2.79	1.72	0.73	0.45	20.08
APM-7	1.55	1.47	2.38	2.87	1.72	0.68	0.41	19.66
APM-8	1.50	1.47	2.41	2.81	1.72	0.69	0.43	19.68
APM-9	1.60	1.39	2.28	3.23	1.88	0.67	0.36	19.74
APM-10	1.72	1.34	2.23	3.64	2.11	0.64	0.30	20.58
APM-11	1.45	1.46	2.41	2.39	1.43	0.54	0.47	18.49
APM-12	1.19	1.45	2.26	1.86	1.59	1.18	0.49	19.37
APM-13	1.20	1.49	2.30	1.76	1.52	1.14	0.51	19.29
APM-14	1.19	1.55	2.65	1.88	1.50	1.03	0.51	19.31
APM-15	1.22	1.50	2.20	1.81	1.58	1.23	0.49	19.67
APM-16	1.29	1.39	2.22	2.21	1.59	0.95	0.42	18.49
APM-17	1.27	1.47	2.41	2.09	1.55	0.97	0.48	19.21
APM-18	1.24	1.44	2.27	2.05	1.69	1.19	0.47	19.55
APM-19	1.41	1.55	2.23	2.27	1.72	1.13	0.44	20.41
APM-20	1.41	1.43	2.29	2.57	1.75	0.92	0.42	19.51
N-free	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.22

[1]All values reported as percentage on a DM basis. Samples were analyzed in duplicate.

[2]Animal protein meals (APM) as described in Table 4.1.

[3]Total = 23 amino acids including hydroxylysine, hydroxyproline, lanthionine, ornithine, and taurine.

Table 4.4 Standardized ileal amino acid digestibility of semi-purified diets containing animal protein meals fed to broilers from 25 to 30 d of age[1]

	SID of indispensible amino acids (%)									
Item	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Val
APM-1	78.0	51.6	72.6	72.8	74.4	74.0	75.4	76.5	71.5	72.8
APM-2	77.9	63.5	73.6	76.5	77.1	75.6	76.8	77.2	73.2	75.1
APM-3	77.6	52.4	71.5	74.1	74.7	73.5	72.5	75.9	70.9	73.4
APM-4	79.8	53.8	75.9	76.0	77.3	75.9	78.4	77.8	73.9	75.6
APM-5	83.2	61.1	82.0	84.6	84.8	83.6	85.5	85.3	81.5	83.5
APM-6	83.8	58.6	80.5	83.6	83.8	84.2	84.9	84.8	80.2	81.5
APM-7	83.3	67.4	81.9	84.1	85.0	82.0	83.4	85.0	81.3	83.6
APM-8	81.0	61.7	79.6	81.2	81.9	80.5	82.4	81.9	78.2	79.4
APM-9	79.7	68.5	82.7	82.7	83.1	81.9	82.9	82.5	80.3	81.8
APM-10	71.6	76.2	78.1	77.3	77.7	76.2	78.7	74.4	75.6	76.3
APM-11	59.4	32.0	42.3	43.8	46.5	44.5	41.4	49.2	42.1	45.1
APM-12	72.3	48.4	67.2	70.1	68.1	74.2	75.0	69.8	63.1	65.6
APM-13	71.3	46.6	66.1	70.0	67.4	70.8	72.9	68.7	62.4	65.7
APM-14	83.0	61.4	73.5	79.0	79.5	75.0	76.5	81.6	73.3	77.1
APM-15	74.6	54.9	70.7	74.6	73.5	76.2	72.5	75.3	68.4	72.8
APM-16	75.9	58.3	68.3	73.8	74.0	69.4	71.5	74.9	70.1	72.5
APM-17	80.9	55.5	73.1	77.8	77.6	76.9	76.6	79.3	71.7	76.1
APM-18	75.0	49.6	67.7	76.6	74.5	74.5	71.7	75.9	69.1	73.9
APM-19	78.8	60.5	81.8	80.8	81.1	82.4	79.4	82.0	76.1	79.3
APM-20	71.5	48.1	61.8	70.8	69.8	64.7	66.3	72.0	64.5	68.5
Mean	76.9	56.5	72.5	75.5	75.6	74.8	75.2	76.5	71.4	74.0
SEM[2]	1.5	2.7	1.6	1.7	1.6	1.4	1.3	1.6	2.0	1.8
P-value[3]	$P < 0.001$									

[1]Values represent least squares means of 6 replicate pens with 12 birds per pen at 25 d of age. SID = standardized ileal digestibility. Animal protein meals (APM) as described in Table 4.1.

[2]Pooled standard error.

[3]*P*-value of Analysis of Variance.

Table 4.4 (continued)

	SID of dispensible amino acids (%)						
Item	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
APM-1	76.9	62.2	72.5	75.1	72.4	67.9	73.4
APM-2	75.8	63.4	73.5	71.2	68.6	72.1	75.0
APM-3	75.0	61.1	71.9	71.1	69.8	67.2	72.4
APM-4	76.6	61.8	73.7	71.2	69.1	66.9	76.1
APM-5	81.3	72.7	80.7	76.0	74.0	76.4	82.7
APM-6	82.0	66.8	80.6	76.6	75.5	74.9	83.2
APM-7	81.0	72.0	80.4	75.0	73.7	75.9	81.8
APM-8	78.1	66.1	77.5	72.4	71.3	73.0	81.5
APM-9	77.8	73.5	78.4	72.5	70.1	75.2	81.1
APM-10	69.5	68.2	71.4	64.0	62.1	69.8	76.5
APM-11	56.4	34.1	46.8	59.4	55.8	41.0	43.0
APM-12	68.8	52.1	66.3	65.1	59.7	61.3	67.9
APM-13	68.6	52.2	66.4	65.3	60.0	58.5	67.3
APM-14	75.8	65.7	76.2	67.7	67.5	72.3	79.2
APM-15	70.6	58.4	68.9	66.1	64.8	70.2	72.7
APM-16	71.8	59.2	68.6	67.1	65.5	70.3	72.2
APM-17	75.9	60.5	73.5	69.9	67.7	70.1	74.8
APM-18	70.2	56.9	68.0	64.2	64.0	70.1	72.1
APM-19	75.6	68.0	74.1	67.7	67.0	74.5	79.7
APM-20	65.8	51.7	63.7	60.4	58.5	64.6	68.2
Mean	73.7	61.3	71.7	68.9	66.9	68.6	74.0
SEM[2]	1.6	2.0	1.6	1.9	1.9	1.9	1.8
P-value[3]				$P < 0.001$			

[1]Values represent least squares means of 6 replicate pens with 12 birds per pen at 25 d of age. SID = standardized ileal digestibility. Animal protein meals (APM) as described in Table 4.1.

[2]Pooled standard error.

[3]*P*-value of Analysis of Variance.

Table 4.5 Pepsin digestibility, PC IDEA values, and PC IDEA predicted digestibility of amino acids in animal protein meals[1]

Item	Pepsin digestibility (%)	PC IDEA value	PC IDEA predicted digestibility (%)									
			Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Val
APM-1	87.0	0.373	80.7	34.5	63.7	69.7	72.4	56.5	68.0	79.1	66.2	69.6
APM-2	87.9	0.402	82.0	40.8	66.6	73.3	75.6	62.6	71.8	81.0	69.7	72.3
APM-3	90.0	0.375	80.8	35.0	63.9	70.0	72.6	57.0	68.3	79.2	66.4	69.8
APM-4	90.5	0.383	81.1	36.6	64.6	70.9	73.5	58.6	69.3	79.7	67.3	70.5
APM-5	92.2	0.435	83.5	47.8	70.0	77.3	79.2	69.2	76.1	83.2	73.6	75.5
APM-6	92.3	0.434	83.5	47.4	69.8	77.1	78.9	68.8	75.8	83.1	73.4	75.4
APM-7	92.9	0.478	85.5	56.1	74.3	81.9	83.3	76.5	80.9	86.0	78.2	79.6
APM-8	93.0	0.445	84.0	49.8	71.0	78.4	80.1	70.9	77.2	83.8	74.7	76.5
APM-9	92.7	0.500	86.6	60.0	76.6	84.0	85.2	79.6	83.2	87.5	80.3	81.8
APM-10	95.9	0.463	84.8	53.0	72.8	80.2	81.7	73.7	79.1	85.0	76.5	78.2
APM-11	81.1	0.296	77.1	15.5	55.8	58.4	62.5	36.9	56.3	74.0	55.4	62.3
APM-12	81.1	0.416	82.7	44.0	68.1	75.2	77.2	65.7	73.8	81.9	71.5	73.8
APM-13	82.9	0.413	82.5	43.2	67.7	74.7	76.8	64.9	73.3	81.7	71.0	73.4
APM-14	86.4	0.322	78.3	22.0	58.4	62.3	65.9	43.8	60.3	75.7	59.1	64.7
APM-15	87.2	0.365	80.3	32.5	62.8	68.5	71.3	54.5	66.8	78.5	65.0	68.8
APM-16	88.4	0.378	80.9	35.6	64.2	70.4	73.0	57.6	68.7	79.4	66.8	70.1
APM-17	89.0	0.374	80.7	34.7	63.8	69.8	72.5	56.7	68.1	79.2	66.3	69.7
APM-18	90.3	0.392	81.6	38.9	65.7	72.2	74.6	60.8	70.7	80.4	68.6	71.5
APM-19	88.9	0.397	81.8	39.8	66.1	72.8	75.1	61.7	71.2	80.7	69.1	71.9
APM-20	90.7	0.400	81.9	40.6	66.5	73.2	75.5	62.4	71.7	80.9	69.6	72.2
Mean	89.0	0.402	82.0	40.4	66.6	73.0	75.3	61.9	71.5	81.0	69.4	72.4
SEM[2]	0.3	0.001	0.3	1.3	0.6	0.7	0.7	1.2	0.8	0.4	0.7	0.6
P-value[3]	$P < 0.001$											

[1]Values represent least squares means of 6 replicate samples of each animal protein meal (APM). PC IDEA = Poultry Complete IDEA (Novus International, Inc., St. Charles, MO). Individual APM as described in Table 4.1.

[2]Pooled standard error.

[3]*P*-value of Analysis of Variance.

Table 4.5 (continued)

	PC IDEA predicted digestibility (%)						
Item	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
APM-1	74.9	49.9	70.0	77.0	73.7	67.6	81.4
APM-2	76.8	53.4	72.5	78.6	75.6	70.1	82.9
APM-3	75.0	50.1	70.2	77.1	73.8	67.7	81.5
APM-4	75.5	51.1	70.8	77.6	74.3	68.4	81.9
APM-5	79.0	57.6	75.4	80.4	77.9	73.0	84.7
APM-6	78.9	57.4	75.3	80.4	77.8	72.9	84.7
APM-7	81.9	63.0	79.2	82.8	80.9	76.8	87.1
APM-8	79.7	58.9	76.3	81.0	78.6	73.9	85.3
APM-9	83.4	65.8	81.1	84.0	82.5	78.7	88.3
APM-10	80.9	61.1	77.8	82.0	79.9	75.4	86.3
APM-11	69.7	40.2	63.2	72.8	68.3	60.8	77.1
APM-12	77.8	55.3	73.8	79.4	76.6	71.4	83.7
APM-13	77.5	54.9	73.5	79.2	76.4	71.1	83.5
APM-14	71.4	43.4	65.4	74.2	70.1	63.0	78.5
APM-15	74.3	48.8	69.2	76.6	73.1	66.8	80.9
APM-16	75.2	50.5	70.4	77.3	74.0	68.0	81.6
APM-17	75.0	50.0	70.1	77.1	73.7	67.7	81.4
APM-18	76.2	52.3	71.7	78.1	75.0	69.3	82.4
APM-19	76.5	52.8	72.1	78.3	75.3	69.7	82.7
APM-20	76.7	53.3	72.4	78.5	75.6	70.0	82.9
Mean	76.8	53.5	72.5	78.6	75.7	70.1	82.9
SEM[2]	0.4	0.7	0.5	0.3	0.4	0.5	0.3
P-value[3]				$P < 0.001$			

[1]Values represent least squares means of 6 replicate samples of each animal protein meal (APM). PC IDEA = Poultry Complete IDEA (Novus International, Inc., St. Charles, MO). Individual APM as described in Table 4.1.

[2]Pooled standard error.

[3]*P*-value of Analysis of Variance.

Table 4.6 Pearson correlation coefficients between standardized ileal amino acid digestibility and *in vitro* indicators of amino acid digestibility of animal protein meals

	Item					
	All APM[1]		Bovine, porcine, and mixed species MBM[2]		Animal protein blends[3]	
Item[4]	Pepsin digestibility	PC IDEA[5]	Pepsin digestibility	PC IDEA	Pepsin digestibility	PC IDEA
Arg SID	0.491	0.329	-0.088	0.027	0.537	0.237
P-value	< 0.001	< 0.001	0.525	0.840	< 0.001	0.069
Cys SID	0.644	0.526	0.617	0.537	0.461	0.230
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.079
His SID	0.665	0.617	0.571	0.599	0.488	0.460
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ile SID	0.660	0.595	0.474	0.531	0.615	0.508
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Leu SID	0.698	0.576	0.453	0.521	0.638	0.423
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001
Lys SID	0.582	0.630	0.415	0.506	0.463	0.566
P-value	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
Met SID	0.591	0.638	0.470	0.511	0.400	0.557
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001
Phe SID	0.622	0.461	0.214	0.255	0.631	0.375
P-value	< 0.001	< 0.001	0.103	0.051	< 0.001	0.003
Thr SID	0.714	0.594	0.474	0.512	0.615	0.421
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Val SID	0.699	0.541	0.442	0.477	0.655	0.394
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002

[1]Correlations are based on animal protein meals (APM) 1 to 20 as described in Table 4.1.

[2]Correlations are based on APM 1 to 10 [bovine, porcine, and mixed species meat and bone meal (MBM)].

[3]Correlations are based on APM 11 to 20 (animal protein blends).

[4]SID = standardized ileal digestibility.

[5]Digestibility values predicted from Poultry Complete IDEA (PC IDEA; Novus International, Inc., St. Charles, MO).

Table 4.6 (continued)

	Item					
	All APM[1]		Bovine, porcine, and mixed species MBM[2]		Animal protein blends[3]	
Item[4]	Pepsin digestibility	PC IDEA[5]	Pepsin digestibility	PC IDEA	Pepsin digestibility	PC IDEA
Ala SID	0.527	0.415	-0.108	0.024	0.490	0.302
P-value	< 0.001	< 0.001	0.416	0.856	< 0.001	0.019
Asp SID	0.686	0.560	0.482	0.564	0.548	0.295
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.022
Glu SID	0.617	0.522	0.236	0.329	0.510	0.367
P-value	< 0.001	< 0.001	0.072	0.011	0.004	0.004
Gly SID	0.372	0.309	-0.272	-0.131	0.239	0.156
P-value	< 0.001	< 0.001	0.037	0.321	0.073	0.235
Pro SID	0.481	0.305	-0.201	-0.081	0.465	0.084
P-value	< 0.001	< 0.001	0.126	0.542	< 0.001	0.524
Ser SID	0.649	0.468	0.299	0.395	0.686	0.366
P-value	< 0.001	< 0.001	0.023	0.002	< 0.001	0.004
Tyr SID	0.637	0.637	0.435	0.399	0.550	0.407
P-value	< 0.001	< 0.001	< 0.001	0.002	< 0.001	0.001

[1]Correlations are based on animal protein meals (APM) 1 to 20 as described in Table 4.1.

[2]Correlations are based on APM 1 to 10 [bovine, porcine, and mixed species meat and bone meal (MBM)].

[3]Correlations are based on APM 11 to 20 (animal protein blends).

[4]SID = standardized ileal digestibility

[5]Digestibility values predicted from Poultry Complete IDEA (PC IDEA; Novus International, Inc., St. Charles, MO)

Table 4.7 Multiple linear regression of Poultry Complete IDEA predicted amino acid digestibility and pepsin digestibility on standardized ileal amino acid digestibility of 20 animal protein meals in broilers from 25 to 30 d of age

	Regression coefficient[1]			Statistical parameter[2]		
SIAAD Equation[3]	Intercept	PC IDEA	Pepsin digestibility	R^2	MSE	C(p), Mallows
Arg	4.37	NS	0.82	0.24	31.96	1.13
SE of estimate	12.09	-	0.14	-	-	-
Estimate *P*-value	0.718	-	<0.001	-	-	-
Cys	-80.04	0.20	1.44	0.44	68.99	3.00
SE of estimate	20.92	0.09	0.26	-	-	-
Estimate *P*-value	<0.001	0.026	<0.001	-	-	-
His	-69.22	0.63	1.12	0.51	46.78	3.00
SE of estimate	14.7	0.16	0.21	-	-	-
Estimate *P*-value	<0.001	<0.001	<0.001	-	-	-
Ile	-54.98	0.44	1.10	0.49	44.28	3.00
SE of estimate	14.46	0.13	0.21	-	-	-
Estimate *P*-value	<0.001	<0.001	<0.001	-	-	-
Leu	-65.29	0.38	1.26	0.52	39.35	3.00
SE of estimate	13.46	0.13	0.19	-	-	-
Estimate *P*-value	<0.001	<0.001	<0.001	-	-	-
Lys	-9.65	0.38	0.69	0.46	44.80	3.00
SE of estimate	15.98	0.08	0.21	-	-	-
Estimate *P*-value	0.547	<0.001	0.001	-	-	-
Met	-35.95	0.62	0.75	0.47	45.45	3.00
SE of estimate	14.95	0.12	0.21	-	-	-
Estimate *P*-value	0.018	<0.001	<0.001	-	-	-
Phe	-46.20	NS	1.37	0.39	45.89	2.69
SE of estimate	14.49	-	0.16	-	-	-
Estimate *P*-value	0.002	-	<0.001	-	-	-
Thr	-77.55	0.39	1.37	0.55	42.75	3.00
SE of estimate	14.26	0.13	0.20	-	-	-
Estimate *P*-value	<0.001	0.003	<0.001	-	-	-
Val	-72.60	0.33	1.38	0.51	41.88	3.00
SE of estimate	13.84	0.16	0.20	-	-	-
Estimate *P*-value	<0.001	0.037	<0.001	-	-	-

[1]PC IDEA = Poultry Complete IDEA (Novus International, Inc., St. Charles, MO). NS = not significant ($P > 0.15$).

[2]R^2 is the coefficient of determination; MSE is the mean squared error from the Analysis of Variance for the regression equation; and C(p) is the Mallow's statistic.

[3]SIAAD = Standardized ileal amino acid digestibility.

Table 4.7 (continued)

SIAAD Equation[3]	Regression coefficient[1] Intercept	PC IDEA	Pepsin digestibility	Statistical parameter[2] R^2	MSE	C(p), Mallows
Ala	-7.71	NS	0.92	0.28	33.19	3.06
SE of estimate	12.32	-	0.14	-	-	-
Estimate *P*-value	0.53	-	<0.001	-	-	-
Asp	-80.78	0.34	1.39	0.50	50.17	3.00
SE of estimate	16.05	0.13	0.22	-	-	-
Estimate *P*-value	<0.001	0.012	<0.001	-	-	-
Cys	-80.04	0.20	1.44	0.44	68.99	3.00
SE of estimate	20.92	0.09	0.26	-	-	-
Estimate *P*-value	<0.001	0.026	<0.001	-	-	-
Glu	-47.46	0.41	1.00	0.41	40.61	3.00
SE of estimate	13.68	0.17	0.20	-	-	-
Estimate *P*-value	<0.001	0.016	<0.001	-	-	-
Gly	15.82	NS	0.60	0.14	33.87	2.29
SE of estimate	12.45	-	0.14	-	-	-
Estimate *P*-value	0.206	-	<0.001	-	-	-
Pro	-6.37	NS	0.82	0.23	34.24	1.01
SE of estimate	12.52	-	0.14	-	-	-
Estimate *P*-value	0.61	-	<0.001	-	-	-
Ser	-62.45	NS	1.47	0.42	45.72	2.37
SE of estimate	14.49	-	0.16	-	-	-
Estimate *P*-value	<0.001	-	<0.001	-	-	-
Tyr	-93.80	0.68	1.25	0.43	53.07	3.00
SE of estimate	20.15	0.31	0.23	-	-	-
Estimate *P*-value	<0.001	0.028	<0.001	-	-	-
Ala	-7.71	NS	0.92	0.28	33.19	3.06
SE of estimate	12.32	-	0.14	-	-	-
Estimate *P*-value	0.53	-	<0.001	-	-	-
Asp	-80.78	0.34	1.39	0.50	50.17	3.00
SE of estimate	16.05	0.13	0.22	-	-	-
Estimate *P*-value	<0.001	0.012	<0.001	-	-	-

[1]PC IDEA = Poultry Complete IDEA (Novus International, Inc., St. Charles, MO). NS = not significant ($P > 0.15$).

[2]R^2 is the coefficient of determination; MSE is the mean squared error from the Analysis of Variance for the regression equation; and C(p) is the Mallow's statistic.

[3]SIAAD = Standardized ileal amino acid digestibility.

V. CONCLUSIONS

Animal protein meals are important sources of AA for poultry, but are variable in AA quality. Ileal AA digestibility values of APM are used in diet formulations. To determine IAAD, test ingredients are fed in a SP diet. It has not been determined if differences in ROP exist between CSM diets and SP diets in AA digestibility assays. Due to cost and time constraints, the IAAD assay is not a feasible method for routine evaluation of APM. Thus, prediction equations for IAAD of APM using *in vitro* assays would be beneficial for poultry nutritionists.

The first experiment was designed to determine the effects of diet type and ingredient composition on ROP in broilers. It was concluded that no differences existed in ROP between SP or CSM diets containing MBM. This finding provides further confidence in AA digestibility values for MBM determined with the IAAD assay. On the other hand, T50 and MRT indicated faster ROP in broilers fed SP-DDGS than those fed CSM-DDGS. Consequently, depending on ingredient type and inclusion, the ROP of SP diets may differ from that of CSM diets in broilers. However, it has not been determined if the observed differences in ROP may impact nutrient utilization and broiler performance.

The second experiment evaluated PC IDEA and pepsin digestibility as *in vitro* predictors of SIAAD for 20 APM in broilers. Correlation and multiple linear regression indicated significant relationships of PC IDEA and pepsin digestibility with SIAAD. The

SIAAD of Lys, Met, and Thr were predicted with the following equations: % Lys SIAAD = [–9.65 + (0.38 × % PC IDEA predicted Lys digestibility) + (0.69 × % pepsin digestibility)], (R^2 = 0.46, MSE = 44.80); % Met SIAAD = [–35.95 + (0.62 × % PC IDEA predicted Met digestibility) + (0.75 × % pepsin digestibility)], (R^2 = 0.47, MSE = 45.45). % Thr SIAAD = [–77.5 + (0.39 × % PC IDEA predicted Thr digestibility) + (1.37 × % pepsin digestibility)] (R^2 = 0.55, MSE = 42.75). Values of R^2 for equations to predict SIAAD of Cys, Val, and Ile were 0.44, 0.51, and 0.49, respectively. The range in SIAAD of the APM was limited, and goodness of fit of the prediction equations may have been improved with more APM in the range of 40 to 60 % SIAAD.