EVALUATION OF SEVERAL METHODS FOR DETECTING DIFFERENCES IN AMINO ACID DIGESTIBILITY AMONG MEAT AND BONE MEALS

BY

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THESIS

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ABSTRACT

Digestibility or bioavailability of amino acids (AA) can vary greatly among meat and bone meals (MBM). Five methods were evaluated to determine AA digestibility or bioavailability among MBM samples; these were the pepsin nitrogen digestibility, the precision-fed cecectomized rooster AA digestibility, the precision-fed chick ileal AA digestibility, the standardized ileal amino acid chick digestibility, and the slope-ratio chick growth lysine bioavailability assays. Pepsin nitrogen digestibility was determined using two different pepsin concentrations, 0.02 and 0.002%. The precision-fed cecectomized rooster assay consisted of tube-feeding the test feed ingredient followed by quantitatively collecting excreta for 48 hours. When 16 different MBM samples were evaluated using this assay, AA digestibility values varied among samples and rooster AA digestibility values were significantly correlated with pepsin values; however, most of the variation was due to the two highest and two lowest digestibility samples. The latter indicated that the pepsin assay was sensitive only for detecting large differences among MBM samples. The precision-fed chick ileal AA assay was similar to the rooster assay except that at four hours post-feeding, chicks were humanely euthanized and digesta from the ileum were collected and analyzed for AA. When comparing the precision-fed rooster and chick assays for the two MBM with the highest and two lowest rooster digestibility values, the results confirmed a large difference in AA digestibility among the samples and the two assays were in general agreement. Four additional MBM varying in pepsin digestibility were then obtained and evaluated using the rooster assay and the standardized ileal AA chick assay wherein 16-day-old broiler chicks were ad libitum fed a test diet containing a MBM as the only source of dietary protein for a period of five days. The chicks were then humanely euthanized and digesta from the ileum were collected and analyzed for AA. A slope-ratio chick

growth assay was also conducted wherein week-old chicks were fed a Lys deficient basal diet supplemented with two levels of crystalline test Lys or two levels of the four MBM. Results were in general agreement when comparing the pepsin nitrogen digestibility, the precision-fed cecectomized rooster, the standardized ileal AA digestibility chick, and the slope-ratio chick growth assay. However, results for the chick growth assay were less consistent than the results for the other three assays.

DEDICATION

This thesis is dedicated to all of my friends and family who have supported and believed in me throughout my life.

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LITERATURE REVIEW

INTRODUCTION

Meat and bone meal (MBM) is an important and widely used feed ingredient in poultry diets. It is a particularly good source of amino acids and phosphorus. However, it is well known that the protein quality of MBM can vary greatly among samples (Summers et al., 1964; Boorman, 1992). Digestibility of amino acids in MBM can vary substantially for poultry (Sibbald, 1986; Parsons et al., 1997). Types of processing systems, processing pressure, processing temperature and processing time are all factors that have been shown to affect amino acid digestibility of MBM (Skurray and Herbert, 1974; Batterham et al., 1986; Johnson et al., 1998; Wang and Parsons, 1998). Quality control methods are needed to help manufacturers of animal protein meals to produce meals of consistent nutritional quality (Johnston and Coon, 1979a). A common laboratory method used to monitor or predict amino acid digestibility for MBM is the pepsin digestibility assay. There has been little research done in the last 20 years using the pepsin digestibility assay to detect differences in protein quality among MBM samples.

ANIMAL NUTRITION PROTEIN MEALS

Animal protein meals are vital components of many poultry feeding programs. Billions of pounds of animal by-products are produced annually, and several of those are used in poultry rations each year (Muirhead, 1996). Fish and meat and bone meals have been frequently used and feather and hair meals have been studied as substitutes or supplements for increasingly expensive traditional feed proteins (Johnston and Coon, 1979a). Fish meal is currently used only sparingly because of its high price but MBM is still commonly used. Meat and bone meal is produced from animal offal including restaurant grease, plate waste, trimmings and bones, viscera and undigested feed, blood, heads, hooves, hides and dead livestock, all of which are

unfit for human consumption (Shirley and Parsons, 2001). It is a valuable source of energy and provides essential amino acids, such as lysine and threonine, minerals, and B vitamins for all classes of poultry (Perai et al., 2010).

Rendering a mixture of raw materials can result in major differences in the final chemical makeup of an animal protein meal. Meat and bone meal is processed at different facilities, temperatures, and times causing possible variations among samples or batches. Differences in protein quality of MBM can also be caused by discrepancies in the amino acid content of the raw materials (Eastoe and Long, 1960) and/or variation in amino acid digestibility or bioavailability. Protein quality is characterized by the ability of a feedstuff to supply essential amino acids relative to an animal's metabolic needs (Boorman, 1992). Because amino acid content and digestibility/bioavailability are the primary factors affecting protein quality of MBM, the various methods of determining amino acid digestibility are discussed and reviewed in the following section.

METHODS FOR DETERMINING AMINO ACID DIGESTIBILITY

GROWTH ASSAYS

In vivo methods are very common methods for determining amino acid digestibility or bioavailability. They are categorized as either direct or indirect methods. Microbiological assays, insect assays, and plasma amino acid assays are indirect methods of determining amino acid bioavailability (Sibbald, 1987; Parsons, 2002). Direct types of in vivo methods include growth or growth-type assays in which the response parameter is something other than growth. Growth assays are based on the idea that the amino acid in a protein or feedstuff will have the ability to provide a specific amino acid in supporting growth, a representation of protein accretion (Ravindran and Bryden, 1999). Growth, which is different from body weight gain, is

rarely measured in the assay. The common response criteria include body weight gain, body weight gain as a function of body weight, gain:feed ratio, feed:gain ratio, and nitrogen retention (Sibbald, 1987). These growth assays usually involve the slope-ratio method. Calculations for this assay assume mean responses are plotted against levels of supplementation and the points lie approximately on a series of straight lines, one for the standard and one for each test supplement, and when the lines are deduced down to zero level of supplementation, they all intersect at a common response (Carpenter et al., 1972). Multiple regression analysis is usually used to calculate bioavailability, which is based on the ratio of the slopes of the growth lines for the test feedstuff and amino acid of interest (Sasse and Baker, 1973; Parsons, 2002). Digestion, absorption, and utilization of the amino acid are included in the measurement of the growth response to the dietary amino acid levels so it is favorable method. However, growth assays are expensive, time consuming, are capable of measuring only one amino acid at a time, and often require expensive purified or semi-purified diets (Ravindran and Bryden, 1999).

DIGESTIBILITY ASSAYS

The most common techniques used for estimating amino acid bioavailability are digestibility assays. According to Lemme et al. (2004), digestibility is defined as the fraction of a nutrient ingested that is absorbed by the bird. These assays have the ability to measure all amino acids at once. Excreta (feces and urine) voided from the animal or digesta from the ileum in poultry are collected for digestibility assays. Excreta assays are based on the principle of measuring amino acids that are voided in the excreta, which are then subtracted from dietary amino acids consumed. Studies have shown that the amino acid content of urine is small and has little effect on amino acid digestibility values, even though feces and urine are collected together (Terpstra, 1978; Ravindran and Bryden, 1999). Since hen eggs can break, causing contamination

of the excreta sample, adult roosters are the preferred poultry to conduct digestibility research with. One of the most frequently used assays is the precision-fed cecectomized rooster assay.

PRECISION-FED CECECTOMIZED ROOSTER ASSAY

The precision-fed rooster assay is a rapid feeding assay which was first created to determine true metabolizable energy of feedstuffs, but it is also commonly used to determine amino acid digestibility (Sibbald, 1976). Birds are fasted 24-48 hours prior to feeding a measured quantity of sample and then excreta are then quantitatively collected over a period of 48 hours and analyzed for amino acids. There is a major advantage to this assay because many feed ingredients can be tested in a relatively short time with few birds and the roosters can be used for several assays (Lemme et al., 2004). Calculating endogenous amino acid losses in the precision-fed rooster assay is done by either collecting excreta from fasted roosters or precision-feeding a protein-free diet (Ravindran and Bryden, 1999). Correcting for endogenous losses allows for a true or standardized digestibility coefficient to be determined. True digestibility measures the relative disappearance of an amino acid from the digestive tract in relation to amino acid intake from a particular feedstuff and endogenous excretion. However, there has been some discussion on whether intact roosters and excreta assays can correctly estimate amino acid digestibility due to the microbial fermentation that occurs in the avian ceca (Bryden et al., 1990).

Most of the microbial fermentation occurring in avian species occurs in the ceca. This is the site where the majority of the microorganisms in the poultry intestine may degrade any undigested dietary amino acids for utilization (Parsons, 1986; Mead, 1989). It has been reported that microbial protein possibly contributes approximately 25% of the total excreta analysis (Parsons et al., 1982). Therefore, microbial degradation of amino acids is reduced by removing the ceca. Consequently, amino acid digestibility is more accurately determined because the

composition of undigested feed is relatively unchanged upon excretion (McNab, 1973; Dingle and McNab, 1985; Parsons, 1986; Sibbald, 1987). In a study conducted by Parsons (1986), amino acid digestibility values of MBM determined by the precision-fed cecectomized rooster assay were lower than those determined with conventional birds. The cecetomized rooster amino acid digestibility values were also in accordance with bioavailability values determined by chick growth assays conducted in the same study. Therefore, the study provided strong evidence to suggest that the ceca influenced amino acid and energy excretion and the use of conventional birds in digestibility trials may result in overestimation of amino acid availability (Parsons, 1986).

ILEAL DIGESTIBILITY ASSAYS

Payne et al. (1968) suggested using an ileal digesta collection method to measure amino acid digestibility more accurately as an alternative to excreta assays. This method involves feeding test diets ad libitum. This can be an advantage because it follows a normal feeding pattern and birds of different ages can be used (Garcia et al., 2007). The most commonly used technique involves slaughtering the animal and collecting the contents of the entire ileal region (Adedokun et al., 2007, 2008; Ravindran and Bryden, 1999). Birds are fed a test diet containing the test ingredient as the only source of dietary protein over a period of time, humanely euthanized, and digesta in the ileum from the Meckel's diverticulum to the ileo-cecal junction are collected. Amino acid digestibility is then calculated based on the amino acid concentrations in the diet and ileal digesta and by using a digesta marker (discussed below). Values are usually standardized by correcting for basal endogenous amino acid losses from birds fed a N-free diet or a highly digestible ingredient such as casein (Adedokun et al., 2008). This assay is commonly referred to as the standardized ileal amino acid digestibility (SIAAD) chick assay. One

downside to the slaughter technique is that it allows only one measurement per animal (Sibbald, 1987). Some researchers have suggested using ileal cannulation because the slaughter technique involves sacrificing many birds. This is based on the method of Raharjo and Farrell (1984) in which a simple glass cannula is inserted in the terminal ileum of adult cockerels and used to obtain samples of digesta to measure the disappearance of amino acids in feeds. This method was found to be simple and practical when measuring the amino acid digestibility in feedstuffs for adult roosters trained to consume their daily feed allowance in 1 h. Amino acid digestibility values in ileal cannulated chickens have been reported to be significantly lower for all amino acids in comparison to amino acid digestibility values determined using the ileal slaughter method (Johns et al., 1986). The ileal cannulation method has several disadvantages. It is time-consuming, requires surgery, is difficult to keep the digesta flowing through the cannula, can be used long-term only with adult birds, and it has been suggested that physical alteration to the intestine may interfere with normal physiological processes of the animal (Ravindran and Bryden, 1999; Tanksley et al., 1981). Therefore, the ileal cannulation method has been used very little, and the most common method used to obtain ileal contents is the slaughter technique.

PRECISION-FED ILEAL AMINO ACID DIGESTIBILITY ASSAY

Although frequently used, the precision-fed cecectomized rooster assay and the ileal digestibility assay discussed above have limitations. For example, the rooster assay requires surgery to remove the ceca and it may not be accurate as compared with data collected from very young birds (Garcia et al., 2007). The ileal assay is time-consuming and requires a substantial amount of test feed ingredient. A recent study developed a new bioassay using precision fed 3-wk-old chicks to measure ileal amino acid digestibility (Kim et al., 2011). This assay allows for smaller amounts of a test sample to be used when crop-intubating or tube-feeding chicks. Chicks

are fasted for at least 8 hours, precision-fed approximately 10 g of feed, and ileal digesta is collected at approximately 4 hours post-feeding. This new precision-fed chick assay may provide a tool to assist in determining whether reported differences in amino acid digestibility between the cecetomized rooster and chick ileal assays (Garcia et al., 2007) are due to methodology (i.e., tube-feeding vs. ad libitum consumption) or bird age (adult roosters vs. chicks) because both roosters and chicks can be tube-fed to eliminate the feeding method variable (Kim et al., 2011).

DIGESTIBILITY MARKERS

Test diets in ileal digestibility assays require the use of indigestible marker substances which do not affect nutrient digestibility and have a high recovery rate of almost 100% (Lemme et al., 2004). Ileal digestibility assays require the use of an indigestible marker to relate the amino acid contents in the ileum to those in the diet. The ratio of the concentration of the marker in the diet to the concentration in the ileal digesta or feces is used to calculate amino acid digestibility. The most commonly used markers for digestibility assays are chromic oxide (Cr_2O3), acid insoluble ash (AIA), and titanium dioxide (TiO_2). Chromic oxide is one of the most frequently used indigestible markers. It is effective as an indigestible marker because it can be added at very small (0.25-0.50%) inclusion rates. Chromic oxide is non-toxic to animals and can range from light green to dark green in color (Kotb and Luckey, 1972). Titanium dioxide is becoming a more frequently used marker as more labs develop the ability to analyze for titanium and because titanium dioxide is considered a safer substance to work with (Lemme et al., 2004). Acid insoluble ash, usually Celite® (clay) or silica, can be added to a diet or feed sample and can be recovered in the feces because it is not absorbed in the gastrointestinal tract. Scott and Hall

(1998) reported that an acid insoluble ash marker, as compared to chromic oxide, provided a more accurate estimate of feeding value.

ESTIMATING PROTEIN QUALITY BY IN VITRO METHODS

Amino acid digestibility can also be estimated using in vitro methods. Chemical, microbiological, and near infrared reflectance (NIR) spectroscopy in vitro assays are valuable for their simplicity and rapid turnaround time. Most in vitro assays are based on measurements of free epsilon amino groups of lysine in proteins or on release of amino acids from proteins when they are incubated with proteolytic enzymes (Parsons, 2002). These methods are able to be duplicated and require no animal use, which is favored by many institutions due to increasing pressure to reduce or cease animal use in research. In vitro assays can provide useful information regarding heat damaged proteins and on relative ranking of different protein sources or samples of a given protein source (Ravindran and Bryden, 1999). This can especially affect lysine because it is the second limiting amino acid in most practical poultry diets, and its ε-amino group is highly susceptible to the Maillard reaction during heat treatment.

Dye binding is a very rapid assay that involves a protein-dye complex that has a high extinction coefficient thus leading to great sensitivity in measurement of the protein (Bradford, 1976). A study done by McFarland and Coon (1984) used a Coomassie brilliant blue dye-binding assay and found it to be an inexpensive method of determining protein digestibility in vivo. The assay was able to determine the percentage of the sample protein that is actually digested and absorbed in vivo. However one study reported that the dye-binding capacity of animal protein meals does not serve as an adequate indicator of protein quality, but it can be used to determine protein quantity (Johnston and Coon, 1979a).

Another rapid assay is the multienzyme pH change assay. This assay is an advantageous in vitro method for predicting protein digestibility because it can be completed within one hour with a high degree of sensitivity (Hsu et al., 1977). It is a multienzyme system consisting of trypsin, chymotrypsin and peptidase. Test ingredients were incubated in an aqueous suspension with the multienzyme system and the pH was adjusted to 8.0. When the multienzyme solution was added to the protein suspension, a rapid decline in pH was observed due to the freeing of amino acid carboxyl groups from the protein chain by the proteolytic enzymes. Results of Hsu et al. (1977) suggested that the pH at 10 min following the addition of the proteolytic enzymes was a good index for predicting the apparent digestibility of protein in rats.

PEPSIN DIGESTIBILITY ASSAY

The most common in vitro assay used for animal protein meals is the pepsin digestibility assay. This assay is based on enzymatic digestion of protein to yield a rapid estimate of protein digestibility or quality. The concentration of pepsin has been found to play a critical role in the assay. Reports have stated that it is important that the Association of Official Analytical Chemists' (AOAC) (1975, 1980) recommendation of a 0.2% level of pepsin be reduced to 0.002 or 0.0002% to increase the sensitivity of the assay (Johnston and Coon, 1979a; Johnston and Coon, 1979b; Han and Parsons, 1991; Parsons et al., 1997). Even poorly digestible proteins can be digested almost completely within the 16-hr digestion period when using the large quantity of 0.2% pepsin currently suggested by the AOAC method (Ambrose and Snyder, 1964; Dreosti et al., 1964; Lovern, 1964; Lovern et al., 1964). A study by Johnston and Coon (1979a) showed that digestible N values for nine meat meals at a 0.02% pepsin concentration were almost identical to those values with 0.2% pepsin, but when the digestible N values were reduced to 0.002%, the range in values among the samples increased substantially. Parsons et al. (1997)

also studied the pepsin digestibility assay of 14 MBM samples using 0.2, 0.002 or 0.0002% pepsin. Pepsin digestibility for the 14 samples ranged from 83-89, 54-83 and 29-61%, respectively, for the 0.2, 0.002 and the 0.0002% pepsin levels. Therefore, reducing the pepsin concentration reduced the digestibility values and increased the variation among samples. The correlation between cecectomized rooster Lys digestibility values and pepsin digestibility values was also much higher for 0.002 and 0.0002% pepsin than for 0.2% pepsin ($r = 0.69$ and 0.60 vs 0.25, respectively).

A study conducted by Han and Parsons (1991) evaluated protein and amino acid quality of seven commercial feather meal (FM) samples using the pepsin digestibility assay. Pepsin digestibility values of N in the FM varied from 70.2 to 81.2% with 0.2% pepsin. Digestibility values were substantially lower with 0.002% pepsin at 17.0 to 49.1%. This study proved that 0.002% pepsin produces greater difference in digestibility estimates among FM samples than does 0.2% pepsin and further verified that using 0.002% pepsin is more sensitive in detecting in vivo quality difference among FM (Han and Parsons, 1991).

SUMMARY AND THESIS OBJECTIVES

Meat and bone meal quality can vary greatly among samples due to chemical makeup and processing factors. The pepsin digestibility assay is one of the most common in vitro assays used to determine protein and amino acid quality in MBM. The cecectomized rooster assay and the standardized ileal chick assay are the most widely used and accepted methods of determining digestible amino acids in poultry feed ingredients such as MBM. The precision-fed ileal amino acid digestibility method in chicks also seems to be a promising method for determining digestible amino acids. Very little research has been conducted to evaluate the pepsin digestibility assay for MBM during the last 20 years. Commercial processing equipment and

processing methods for MBM have been modified and improved during this period in attempt to produce more consistent high quality MBM (Darling International, personal communication). In addition, many commercial buyers of MBM make purchasing decisions (such as accepting or rejecting and/or discounting) based on small differences in pepsin digestibility among MBMs (Darling International, personal communication). The latter is of concern and may not be justified since the earlier studies by the Johnston and Coon (1979b) and Parsons et al. (1997) labs concluded that the pepsin assay was particularly useful for detecting large or substantial differences in quality among MBM samples, not small differences. Therefore, the current thesis was conducted to re-evaluate the pepsin digestibility for detecting differences in amino acid digestibility among MBM produced in current commercial plants. Two different pepsin concentrations were evaluated and the pepsin N digestibility values were compared to in vivo amino acid digestibility values determined using the precision-fed cecectomized rooster excreta assay, the precision-fed chick ileal assay, and the ileal SIAAD assay.

LITERATURE CITED

Adedokun, S.A., C.M. Parsons, M.S. Lilburn, O. Adeola, and T.J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590-2597.

Adedokun, S.A., O. Adeola, C.M. Parsons, M.S. Lilburn, and T.J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535-2548.

Ambrose, M.E., and D.G. Snyder. 1964. Some studies in the U.S.A. Fishing News Int 3:210-213.

Association of Official Analytical Chemists. 1975. Official Methods of Analysis. 12th Rev. ed. Association of Official Analytical Chemists, Washington, D.C.

Association of Official Analytical Chemists. 1980. Official Methods of Analysis. 13th Rev. ed. Association of Official Analytical Chemists, Washington, D.C.

Batterham, E.S., R.E. Darnell, L.S. Herbert, and E.J. Major. 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal as determined by slope-ratio assays with growing pigs, rats and chicks and by chemical techniques. Br. J. Nutr. 55:441-453.

Boorman, K.N. 1992. Protein quality and amino acid utilization in poultry. pp 51-70 In: Recent Advances in Animal Nutrition. P.C. Garnsworthy, W. Haresign and D.J.A. Cole (ed.). Butterworth-Heinman, Ltd., Boston, MA.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Bryden, W.L., P. Siriwan, and E.F. Annison. 1990. pp 74-278. Developments in the estimation

of amino acid availability. In: Proceedings of the Eigth Australian Poultry and Feed Convention. Gold Coast.

Carpenter, K.J., I. McDonald, and W.S. Miller. 1972. Protein quality of feeding-stuffs. Br. J. Nutr. 27:7-17.

Dingle, J.G., and J.M. McNab. 1985. Colostomy of the fowl. World's Poult. Sci. J. 41:115-123.

Dreosti, G.M., S.G. Wichers, and W.J. Conradie. 1964. Some South African studies. Fishing News Int. 3:314-318.

Eastoe, J.E., and J.E. Long. 1960. The amino-acid composition of processed bones and meat. J Sci. Food Agric. 11:87-92.

Garcia, A.R., A.B. Batal, and N.M. Dale. 2007. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci. 86:94-101.

Han, Y., and C.M. Parsons. 1991. Protein and amino acid quality of feather meals. Poult. Sci. 70:812-822.

Hsu, H.W., D.L. Vavak, L.D. Satterlee, and G.A. Miller. 1977. A multienzyme technique for estimating protein digestibility. J. Fd. Sci. 42:1269-1273.

Johns, D.C., C.K. Low, and K.A.C. James. 1986. Comparison of amino acid digestibility using the ileal digesta from growing chickens and cannulated adult cockerels. British Poult. Sci. 27:679-685.

Johnson, M.L., C.M. Parsons, G.C. Fahey, Jr., N.R. Merchen, and C.G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J. Anim. Sci. 76:1112-1122.

Johnston, J., and C.N. Coon. 1979a. A comparison of six protein quality assays using

commercially available protein meals. Poult. Sci. 58:919-927.

Johnston, J., and C.N. Coon. 1979b. The use of varying levels of pepsin for pepsin digestion studies with animal proteins. Poult. Sci. 58:1271-1273.

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2011. Development of a precision-fed ileal amino acid digestibility assay using 3-week-old broiler chicks. Poult. Sci. 90:396-401.

Kotb, A.R., and T.D. Luckey. 1972. Markers in nutrition. Nutr. Abs. Rev. 42:813-845.

Lemme, A., V. Ravindran and W.L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World Poult. Sci. J. 60:423-437.

Lovern, J.A. 1964. Pepsin digestibility: As an index of quality in fish meals. II. Fishing News Int. 3:310-314.

Lovern, J.A., J. Olley, and R. Pirie, 1964. Pepsin digestibility: As an index of quality in fish meals. II. Fishing News Int 3:310-314.

McFarland, D.C., and C.N. Coon. 1984. The evaluation of by-product protein digestibility utilizing coomassie blue dye-binding. Poult. Sci. 63:2225-2228.

McNab, J.M. 1973. The avian caeca: a review. World's Poult. Sci. 29:251-263.

Mead, G.C. 1989. Microbes of the avian cecum: Types present and substrates utilized. J. Exptl. Zoo Suppl. 3:48-54.

Muirhead, S. 1996. Impact of ruminant-to-ruminant feed ban far reaching. Feedstuffs 68(29):4-5.

Parsons, C.M. 1986. Determination of digestible and available amino acids in meat meal using conventional and caecectomized cockerels or chick growth assays. Br. J. Nutr. 56:227-240.

Parsons, C.M. 2002. Digestibility and bioavailability of protein and amino acids. pp 115-135 In:

Poultry feedstuffs: Supply, composition, and nutritive value J.M. McNab and K.N. Boorman (ed.). CABI, Oxon, UK.

Parsons, C.M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poult. Sci. 76:361-368.

Parsons, C.M., L.M. Potter, R.D. Brown, Jr., T.D. Wilkins, and B.A. Bliss. 1982. Microbial contribution to dry matter and amino acid content of poultry excreta. Poult. Sci. 61:925-932.

Payne, W.L., G.F. Combs, R.R. Kifer, and D.G. Snider. 1968. Investigation of protein quality-ileal recovery of amino acids. Fed. Proc. 27:1199-1203.

Perai, A.H., H. Nassiri Moghaddam, S. Asadpour, J. Bahrampour, and Gh. Mansoori. 2010. A comparison of artificial neural networks with other statistical approaches for the prediction of true metabolizable energy of meat and bone meal. Poult. Sci. 89:1562-1568.

Raharjo, Y., and D.J. Farrell. 1984. A new biological method for determining amino acid digestibility in poultry feedstuffs using a simple cannula, and the influence of dietary fibre on endogenous amino acid output. Anim. Feed Sci. Tech. 12:29-45.

Ravindran, V., and W.L. Bryden. 1999. Amino acid availability in poultry – in vitro and in vivo measurements. Aust. J. Agric. Res. 50:889-908.

Sasse, C.E., and D.H. Baker. 1973. Availability of sulfur amino acids in corn and corn gluten meal for growing chicks. J. Anim. Sci. 37:1351-1355.

Scott, T.A., and J.W. Hall. 1998. Using acid insoluble ash marker ratios (Diet:Digesta) to predict digestibility of wheat and barley metabolizable energy and nitrogen retention in broiler chicks. Poult. Sci. 77:674-679.

Shirley, R.B., and C.M. Parsons. 2001. Effect of ash content on protein quality of meat and bone

meal. Poult. Sci. 80:626-632.

Sibbald, I.R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci. 55:303-308.

Sibbald, I.R. 1986. The T.M.E. system of feed evaluation: methodology, feed composition data, and bibliography. Technical Bulletin 1986-4E. Agriculture Canada, Ottawa, ON, Canada.

Sibbald, I.R. 1987. Estimation of bioavailable amino acids in feedingstuffs for poultry and pigs: A review with emphasis on balance experiments. Can. J. Anim. Sci. 67:221-300.

Skurray, G.R., and L.S. Herbert. 1974. Batch dry rendering: Influence of raw materials and processing conditions on meat meal quality. J. Sci. Fd. Agric. 25:1071-1079.

Summers, J.D., S.J. Slinger, and G.C. Ashton. 1964. Evaluation of meat meal as a protein supplement for the chick. Can. J. Anim. Sci. 44:228-234.

Tanksley, T.D., Jr., D.A. Knabe, K. Purser, T. Zebrowska, and J.R. Corley. 1981. Apparent digestibility of amino acids and nitrogen in three cottonseed meals and one soybean meal. J. Anim. Sci. 52:769-777.

Terpestra, K. 1978. Total and digestible amino acids. pp 97-101. In: Proceedings Second Symposium on Poultry Nutrition, Beekbergen, The Netherlands.

Wang, X., and C.M. Parsons. 1998. Effect of raw material source, processing system, and processing temperatures on amino acid digestibility of meat and bone meals. Poult. Sci. 77:834-841.

Chapter 2

EVALUATION OF THE PEPSIN DIGESTIBILITY ASSAY FOR PREDICTING AMINO ACID DIGESTIBILITY OF MEAT AND BONE MEALS

ABSTRACT

Sixteen meat and bone meal (MBM) samples were obtained and selected from various company plants to provide a wide range in pepsin digestibility values. Pepsin digestibility was determined using either 0.02 or 0.002% pepsin. Amino acid (AA) digestibility of the 16 MBM samples was then determined using a precision-fed cecectomized rooster assay. As expected, the 0.02% pepsin digestibility values were numerically higher than the 0.002% pepsin values. The values varied from 77 to 93% for 0.02% pepsin and from 67 to 91% for 0.002% pepsin. The rooster AA digestibility results showed a wide range of values mostly due to the samples with the two lowest values and the two highest values. The AA digestibility values for the other 12 samples were intermediate and generally similar among samples. A precision-fed chick ileal AA digestibility assay confirmed that there were large differences in AA digestibility among the four samples having the lowest and highest rooster digestibility values. Correlation analyses between pepsin and AA digestibility values showed that the correlation values (r) were generally high and significant for all AA when all 16 MBM samples were included in the analysis. However, when the MBM samples with the two lowest and the two highest rooster digestibility values were not used in the correlation analyses, the correlation coefficient values (r) were generally very low and not significant ($P > 0.05$). The results indicated that the pepsin digestibility assay is only useful for detecting large differences in protein quality among MBM. The pepsin assay was not useful for predicting differences in quality among MBM samples of average or intermediate quality. For example, rooster AA digestibility was similar for MBM samples having 0.02% pepsin digestibility values of 80 to 90% and 0.002% pepsin values of 72 to 86%. There also was

no clear advantage for using 0.02 versus 0.002% pepsin since the correlation values were similar for both.

INTRODUCTION

The composition and digestibility of meat and bone meal can vary greatly among samples. Differences in the nutrient composition and energy values of MBM are largely due to the rendering of various raw materials (Adedokun and Adeola, 2005; Johnston and Coon, 1979a) which makes it difficult to determine nutritive values for feed formulation (Perai et al., 2010). One of the most important concerns of MBM use in poultry and livestock rations is its variability in protein quality (Parsons et al., 1997). Manufacturers and nutritionists need to evaluate protein quality of MBM with a rapid, inexpensive, and accurate method in order to produce meals of high nutritional value consistently (Parsons, 1986).

The pepsin assay (AOAC, 1980) is widely used by the animal feed industry to monitor quality of MBM, particularly for detecting low quality samples. It is a moderately simple, inexpensive, and rapid assay, and many samples can be compared at the same time (Ravindran and Bryden, 1999). Research conducted by Parsons et al. (1997) and Johnston and Coon (1979b) showed that the pepsin digestibility assay was useful for detecting large differences in protein quality among commercial animal meals if the concentration of pepsin was reduced from 0.2% to 0.02, 0.002, or 0.0002%. Very little additional research has been done with the pepsin assay during the last 20 years.

The primary objective of the current study was to reevaluate the pepsin digestibility assay for detecting differences in AA digestibility among MBM produced in current commercial rendering plants. Particular emphasis was on assessing the sensitivity of the pepsin assay for detecting small/moderate differences in AA digestibility versus large differences among MBM

samples. In addition, the AA digestibility of some of the MBM samples were determined in both the precision-fed cecectomized rooster assay and a new precision-fed chick ileal digestibility assay to determine if values were in agreement between assays.

MATERIALS AND METHODS

Meat and Bone Meals

Sixteen MBM samples were obtained from various commercial rendering plants from Darling International, Inc., Irving, Texas. Pepsin nitrogen digestibility was determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 1980) at Darling International Analytical Laboratory in Ankeny, Iowa except that the recommended level of 0.2% pepsin was reduced to 0.02 and 0.002% pepsin concentrations.

Precision-fed Cecectomized Rooster Assay

Cecectomized Single Comb White Leghorn roosters were utilized in the precision-fed rooster assay according to the procedure of Parsons (1985). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. After 26 h without feed, four roosters were tube-fed 30 grams of a MBM sample. The roosters were then placed in a cage with a plastic tray underneath, and the total excreta were collected for 48 hours. The excreta samples were frozen and stored at -20˚C then freeze-dried, ground, and the MBM and dried excreta were analyzed for AA at the University of Missouri. Digestibility of AA was then calculated for each of the 64 roosters.

Precision-fed Chick Ileal Amino Acid Digestibility Assay

The precision-fed ileal AA digestibility assay was conducted using the procedures described by Kim et al. (2011). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. Sixty-four 21-d-old broiler

chicks were fasted overnight then 4 groups of 4 chicks were tube-fed 10 grams of 1 of 4 MBM samples. The 4 MBM samples were the 2 samples that had the lowest and the 2 samples that had the highest AA digestibility values from the rooster assay. Chromic oxide was added to the MBM samples as an indigestible marker at a level 0.30%. Chicks were then placed in a cage and at 4 hours post-feeding, they were euthanized via CO_2 and ileal digesta were collected from the Meckel's diverticulum to the ileal-cecal junction. Ileum contents were pooled, frozen, and stored at -20˚C then freeze-dried, ground by using a mortar and pestle, and the MBM samples and ileal digesta samples were analyzed for AA and chromium at the University of Missouri.

Calculations

Standardized AA digestibility values for the precision-fed cecectomized rooster assay were calculated using the following formula. Amino acids were standardized using an endogenous correction based on AA excretion by fasted roosters.

Standardized AA digestibility, % = [(AA consumed, mg – AA excreted, mg + endogenous AA excreted, mg) / AA consumed, mg] x 100.

Standardized AA digestibility values for the precision-fed chick ileal AA digestibility assay were calculated using the following formula by Moughan et al. (1992).

Apparent ileal AA digestibility = [1 – (chromium in diet/chromium in digesta) x (AA in digesta/AA in diet)] x 100,

Standardized AA digestibility, % = apparent digestibility + [(ileal endogenous AA flow, g/kg of DM intake)/(AA in the diet, g/kg of DM intake)] x 100.

Statistical Analysis

Data from both animal assays were subjected to ANOVA and PROC GLM tests (SAS Institute, 2008) as a completely randomized design. Statistical significance of differences among

individual treatments was then determined using the least significant difference test (Carmer and Walker, 1985). Statistical significance was indicated at $P < 0.05$. Correlations of pepsin nitrogen digestibility with rooster AA digestibility were assessed using Pearson's linear test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

For presentation of the results in Tables 2.1-2.3, the numbering of the MBM samples was based in general accordance with the rooster AA digestibility values, particularly the two lowest and two highest digestibility samples. The total AA concentrations of the 16 MBM samples are presented in Table 2.1. These values varied greatly among samples. The variation in AA concentrations among samples was likely primarily due to variation in raw material composition among samples.

The pepsin nitrogen digestibility values for the 16 MBM samples showed a substantial amount of variation among samples (Table 2.2). As expected, the values for the 0.02% pepsin nitrogen digestibility were numerically lower than the 0.002% pepsin values. The values varied from 77 to 93% for 0.02% pepsin and from 67 to 91% for 0.002% pepsin.

The standardized AA digestibility values for the 16 MBM samples determined by the precision-fed cecectomized rooster assay are shown in Table 2.3. As observed above for pepsin digestibility and AA concentrations, there was a considerable amount of variation in AA digestibility among MBM samples ($P<0.05$). These results generally agreed with previous research reported by Parsons et al. (1997). The difference in AA, particularly cysteine, digestibility among samples may be due primarily to processing effects (Baker et al., 1981; Parsons, 1986).

A large part of the variation among samples was due to the two samples that had the lowest AA digestibility values (Samples 1 and 2) and the two that had the highest AA digestibility values (Samples 15 and 16). Cysteine exhibited the largest difference in digestibility among MBM samples. The AA digestibility values for the other 12 samples were intermediate and more similar among samples. When all 16 MBM samples were included in the correlation analyses between pepsin digestibility and rooster AA digestibility, the correlation values (r) were significant for all amino acids (Table 2.4). However, when the two samples with the lowest pepsin values and the two samples with the highest pepsin values were excluded from the correlation analyses (Table 2.5), the correlation coefficient values (r) were very low and only one of them was significant ($P>0.05$). The relationship between 0.002% pepsin nitrogen digestibility and lysine and cysteine digestibility in roosters is further illustrated in Figures 2.1 and 2.2. Figure 2.1 shows that the line for lysine and cysteine digestibility is essentially flat (zero slope) when the two lowest and highest pepsin MBM samples are not used. Thus, the significant correlation between pepsin digestibility and rooster AA digestibility was mostly due to the two lowest digestibility and the two highest digestibility samples. These results suggest that the pepsin N digestibility assay is sensitive for detecting large differences in AA digestibility among samples, but not small or moderate differences.

When comparing standardized AA digestibility in cecectomized roosters to ileal AA digestibility in chicks for the two lowest pepsin digestibility MBM samples, all AA digestibility values except for cysteine and lysine were significantly higher for roosters than chicks for Sample 1 (Table 2.6). However, for Sample 2, most AA digestibility values were not significantly different between roosters and chicks, and when significant differences did occur, the chick values were usually higher than the rooster values. A comparison of the rooster and

chick standardized AA digestibility values for the two highest pepsin digestibility MBM samples (Samples 15 and 16) are presented in Table 2.7. For Sample 15, digestibility values were significantly different between roosters and chicks for only 3 AA. In contrast, for Sample 16, most AA digestibility values were significantly higher for roosters than chicks. Similar inconsistent differences between precision-fed roosters and chicks as those observed herein were also reported by Kim et al. (2012). However, Kim et al. (2012) reported that AA digestibility values were higher for roosters than chicks for some feed ingredient samples. It was observed in the current study that the rooster values were higher than the chick values for 2 of the 4 MBM samples. As discussed by Kim et al. (2012), the lower values for chicks may be due, at least partly, to collecting ileal digesta from the entire ileal section of the small intestine. Some previous studies have shown that there is AA disappearance from the intestine as digesta moves through the ileum; thus, collecting digesta from the entire ileum may overestimate undigested AA and underestimate AA digestibility (Kadim and Moughan, 1997; Kluth et al., 2005; Rezvani et al., 2008). It is also interesting to note that the variability in AA digestibility values was much greater in the precision-fed chick assay than the precision-fed rooster assay, with the SEM values for the precision-fed chick assay being approximately three times larger than the precision-fed rooster assay. Kim et al. (2012) also reported larger SEM values for the precision-fed rooster assay for one sample of MBM although the differences were not as large as those observed in the current study.

When comparing the AA digestibility values for all 4 MBM samples in Table 2.6 and 2.7, both the rooster and chick assay yielded much lower values for Samples 1 and 2 than Samples 15 and 16. Thus, both assays were sensitive and in agreement for detecting large differences in AA digestibility among MBM samples.

In conclusion, the results of this study show that the pepsin digestibility assay is valuable only for detecting large differences in protein quality among MBM when levels of 0.02% or 0.002% pepsin are used. The pepsin assay is most useful for detecting very poor quality samples and to a lesser extent very high quality samples. Both the precision-fed cecectomized rooster and precision-fed ileal chick assays are acceptable methods for determining and detecting differences in AA digestibility among MBM samples.

LITERATURE CITED

Adedokun, S.A., and O. Adeola. 2005. Apparent metabolizable energy value of meat and bone meal for white pekin ducks. Poult. Sci. 84:1539-1546.

Association of Official Analytical Chemists. 1980. Official Methods of Analysis. 13th Rev. ed. Association of Official Analytical Chemists, Washington, D.C.

Baker, D.H., Blitenthal, R.C., Boebel, K.P., Czarnecki, G.L., Southern, L.L., and Willis, G.M. 1981. Protein-amino acid evaluation of steam-processed feather meal. Poult. Sci. 60:1865-1872.

Carmer, S.G., and W.M. Walker. 1985. Pairwise multiple comparisons of treatment means in agronomic research. J. Agron. Educ. 14:19-26.

Johnston, J., and C.N. Coon. 1979a. A comparison of six protein quality assays using commercially available protein meals. Poult. Sci. 58:919-927.

Johnston, J., and C.N. Coon. 1979b. The use of varying levels of pepsin for pepsin digestion studies with animal proteins. Poult. Sci. 58:1271-1273.

Kadim, I.T., and P.J. Moughan. 1997. Development of an ileal amino acid digestibility for the growing chicken – Effects of time after feeding and site sampling. Br. Poult. Sci. 38:89-95.

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2011. Development of a precision-fed ileal amino acid digestibility assay using 3-week-old broiler chicks. Poult. Sci. 90:396-401.

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91:1350-1355.

Kluth, H., K. Melhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be

sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271-279.

Moughan, P.J., G. Schuttetr, and M. Leenaars. 1992. Endogenous amino acid flow in the stomach and small intestine of the young growing pig. J. Sci. Food Agric. 60:437-442.

Parsons, C.M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers dried grains with solubles. J. Agric. Sci. 104:469-472.

Parsons, C.M. 1986. Determination of digestible and available amino acids in meat meal using conventional and caecectomized cockerels or chick growth assays. Brit. J. Nutr. 56:227-240.

Parsons, C.M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poult. Sci. 76:361-368.

Perai, A.H., H. Nassiri Moghaddam, S. Asadpour, J. Bahrampour, and Gh. Mansoori. 2010. A comparison of artificial neural networks with other statistical approaches for the prediction of true metabolizable energy of meat and bone meal. Poult. Sci. 89:1562-1568.

Ravindran, V., and W.L. Bryden. 1999. Amino acid availability in poultry – in vitro and in vivo measurements. Aust. J. Agric. Res. 50:889-908.

Rezvani, M., H. Kluth, C. Elwert, and M. Rodehutscord. 2008. Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. Br. Poult. Sci. 49:28-36.

SAS Institute. 2008. SAS User's Guide. Statistics. Version 9.2 ed. SAS Inst. Inc., Cary, NC.

Steel, R.G.D. , and J.H. Torrie, 1980. Principles and procedures of statistics. A biometrical approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

TABLES AND FIGURES

Table 2.1. Total amino acid concentrations (%) in the 16 meat and bone meal (MBM) samples, as-fed basis

	MBM Sample Number							
Amino Acid	1	2	3	4	5	6	7	8
Aspartic Acid	4.35	4.37	3.85	3.84	4.66	4.08	4.66	4.38
Threonine	1.87	1.88	1.63	1.62	2.12	1.86	2.14	1.97
Serine	1.98	1.71	1.82	1.82	2.54	1.94	2.28	2.30
Glutamic Acid	6.72	6.91	6.19	6.17	7.34	6.34	7.27	6.84
Proline	3.75	3.69	4.31	4.27	4.60	3.40	3.96	4.25
Alanine	4.13	4.04	3.81	3.84	4.06	3.59	4.02	3.85
Cysteine	0.34	0.36	0.53	0.52	0.85	0.46	0.64	0.79
Valine	2.76	2.76	2.28	2.27	2.99	2.33	2.78	2.84
Methionine	0.91	0.95	0.67	0.66	0.96	0.87	1.08	0.95
Isoleucine	1.87	1.99	1.63	1.62	2.27	1.71	2.19	2.10
Leucine	3.94	4.00	3.28	3.27	4.06	3.51	4.01	3.86
Tyrosine	1.60	1.56	1.21	1.24	1.66	1.45	1.74	1.60
Phenylalanine	2.21	2.21	1.76	1.77	2.31	1.88	2.21	2.18
Lysine	3.00	3.16	2.64	2.60	3.20	3.13	3.39	3.03
Histidine	1.21	1.26	0.89	0.87	1.19	1.20	1.26	1.10
Arginine	3.59	3.61	3.72	3.70	4.07	3.41	3.95	3.84
Tryptophan	0.27	0.28	0.26	0.27	0.24	0.36	0.42	0.35

Table 2.1 continued. Total amino acid concentrations (%) in the 16 meat and bone meal (MBM) samples, as-fed basis

	MBM Sample Number							
Amino Acid	9	10	11	12	13	14	15	16
Aspartic Acid	4.41	4.99	3.52	3.36	3.94	4.11	4.05	4.50
Threonine	2.06	2.21	1.48	1.37	1.65	1.79	1.76	2.01
Serine	2.59	2.18	1.69	1.64	1.72	1.83	1.82	1.93
Glutamic Acid	6.92	7.69	5.65	5.48	6.25	6.42	6.31	6.83
Proline	4.41	4.01	4.04	3.87	3.91	3.73	3.42	3.57
Alanine	3.97	4.28	3.70	3.53	3.93	3.73	3.60	3.80
Cysteine	0.79	0.61	0.37	0.38	0.34	0.42	0.49	0.51
Valine	2.70	3.01	2.09	1.87	2.46	2.36	2.41	2.68
Methionine	0.91	1.07	0.70	0.64	0.79	0.82	0.85	1.08
Isoleucine	2.07	2.23	1.48	1.33	1.68	1.81	1.78	2.14
Leucine	3.82	4.32	2.88	2.68	3.45	3.43	3.51	3.89
Tyrosine	1.57	1.73	1.07	0.98	1.31	1.37	1.33	1.54
Phenylalanine	2.15	2.31	1.61	1.50	1.92	1.87	1.92	2.11
Lysine	2.98	3.70	2.48	2.36	2.97	2.98	3.14	3.61
Histidine	1.08	1.54	0.81	0.75	1.16	1.17	1.17	1.23
Arginine	3.94	4.03	3.38	3.39	3.47	3.49	3.35	3.71
Tryptophan	0.34	0.37	0.29	0.22	0.32	0.37	0.38	0.40

Table 2.2. Pepsin nitrogen digestibility values (%) for the 16 meat and bone meal samples[1]

Sample Number	0.02% Pepsin	0.002% Pepsin
1	77	67
2	77	71
3	83	72
4	81	73
5	87	78
6	83	80
7	88	80
8	87	81
9	88	82
10	88	84
11	91	84
12	91	85
13	91	85
14	90	86
15	90	89
16	93	91

[1]Values are means of triplicate analyses.

Table 2.3. Standardized amino acid digestibility values (%) for the 16 meat and bone meal (MBM) samples determined by the precision-fed cecectomized rooster assay[1]

	MBM Sample Number							
Amino Acid	1	2	3	4	5	6	7	8
Threonine	46.24^{e}	47.40^{e}	71.20^{d}	74.39^{cd}	75.76^{cd}	82.29^{ab}	75.11^{cd}	74.50^{cd}
Cysteine	26.54^{g}	18.78^{g}	42.84^{ef}	50.61^{cdef}	60.01^{abc}	59.99^{abc}	53.08^{cde}	53.90^{bcde}
Valine	51.49^{e}	51.20^{e}	75.83^{d}	77.26^{cd}	80.26^{bc}	82.83^{ab}	76.12^{d}	77.57^{cd}
Methionine	53.16^{i}	53.83^{i}	80.51^{gh}	81.24^{fgh}	83.37^{defg}	87.29^{bc}	82.48^{efgh}	82.98^{efg}
Isoleucine	52.28^{h}	51.91^{h}	78.57^{fg}	79.60^{defg}	82.58^{cd}	84.86^{bc}	78.71^{efg}	79.67^{defg}
Leucine	50.43^{f}	51.34^{f}	78.63^{de}	79.51^{cde}	82.15^{bcd}	85.69^{ab}	79.10^{de}	79.62^{cde}
Phenylalanine	55.98^{f}	55.76^{f}	80.47^{cde}	81.40^{cde}	83.66^{bc}	85.92^{ab}	80.14^{de}	80.77^{cde}
Lysine	39.71^{f}	39.82^{f}	69.54^{cde}	68.87^{de}	74.71^{bc}	77.13^{ab}	70.09^{cde}	71.30^{cd}
Histidine	47.45^{d}	47.41^{d}	72.44^{c}	71.85^{c}	74.63^{bc}	78.31^{ab}	72.86^{c}	72.74^{c}
Arginine	61.89^{f}	61.94^{f}	81.33^{cd}	83.10^{abc}	84.63^{abc}	85.11^{abc}	82.40^{abc}	81.57^{bcd}
Tryptophan	71.28^{i}	76.61^{h}	81.79^{g}	87.79^{def}	82.24^{g}	91.83^{abc}	89.48^{cde}	88.22^{cdef}

[a-i] Values within a row with no common superscripts are significantly different (P<0.05).

[1]Values are the means of four cecectomized roosters.

Table 2.3 continued. Standardized amino acid digestibility values (%) for the 16 meat and bone meal (MBM) samples determined by the precision-fed cecectomized rooster assay[1]

	MBM Sample Number								
Amino Acid	9	10	11	12	13	14	15	16	Pooled SEM
Threonine	75.36^{cd}	78.12^{bc}	78.71^{bc}	71.70^{d}	71.73^{d}	78.99^{bc}	84.56^{a}	86.09^{a}	1.86
Cysteine	54.53^{bcd}	50.90^{cde}	56.51^{abcd}	38.21^{f}	46.94^{def}	58.66^{abc}	66.74^{a}	65.04^{ab}	3.99
Valine	76.90^{cd}	80.01^{bc}	80.44^{bc}	74.33^{d}	77.36^{cd}	80.78^{bc}	85.82^{a}	86.69^{a}	1.46
Methionine	81.58^{efgh}	85.95^{cd}	84.03^{def}	81.45^{fgh}	79.96^{h}	84.47^{cde}	89.64^{ab}	90.59^{a}	1.05
Isoleucine	79.94^{defg}	81.98^{cde}	82.09^{cdef}	77.94^{g}	79.51^{defg}	82.89^{cd}	87.66^{ab}	88.93^{a}	1.28
Leucine	79.51^{cde}	82.28^{bcd}	82.18^{bcd}	77.71^{e}	79.05^{de}	82.96^{bc}	88.14^{a}	88.23^{a}	3.63
Phenylalanine	80.37^{cde}	83.05^{bcd}	82.33^{cd}	78.81^{e}	80.02^{de}	83.83^{bc}	87.92^{a}	87.81^{a}	3.02
Lysine	71.22^{cd}	74.10^{bc}	68.57^{de}	69.32^{cde}	65.04^{e}	73.94^{bcd}	80.70^{a}	78.33^{ab}	2.00
Histidine	72.34^{c}	74.10^{bc}	74.75^{bc}	70.04^{c}	70.02^{c}	72.88^{c}	81.43^{a}	81.31^{a}	6.72
Arginine	81.26^{cd}	84.93^{ab}	81.62^{bcd}	78.96^{de}	76.84^{e}	85.92^{a}	85.23^{ab}	84.52^{abc}	1.36
Tryptophan	89.21^{cdef}	88.75^{cdef}	90.80^{bcd}	86.65^{ef}	85.54^{gf}	90.33^{bcde}	93.67^{ab}	94.74^{a}	1.36

[a-i] Values within a row with no common superscripts are significantly different (P<0.05).

[1]Values are the means of four cecectomized roosters.

Table 2.4. Correlation of pepsin nitrogen digestibility with individual amino acid digestibility when all 16 meat and bone meal samples are included in the correlation analysis

	0.02% Pepsin Digestibility		0.002% Pepsin Digestibility	
Amino Acid	r	P value	r	P value
Threonine	0.74	<0.0001	0.76	<0.0001
Cysteine	0.63	<0.0001	0.64	<0.0001
Valine	0.75	<0.0001	0.75	<0.0001
Methionine	0.77	<0.0001	0.76	<0.0001
Isoleucine	0.77	<0.0001	0.75	<0.0001
Leucine	0.76	<0.0001	0.74	<0.0001
Phenylalanine	0.74	<0.0001	0.72	<0.0001
Lysine	0.70	<0.0001	0.70	<0.0001
Histidine	0.71	<0.0001	0.70	<0.0001
Arginine	0.64	<0.0001	0.62	<0.0001
Tryptophan	0.71	<0.0001	0.78	<0.0001

Table 2.5. Correlation of pepsin nitrogen digestibility with individual amino acid digestibility when meat and bone meal Samples 1, 2, 15, and 16 are excluded from the correlation analysis

	0.02% Pepsin Digestibility		0.002% Pepsin Digestibility	
Amino Acid	r	P value	r	P value
Threonine	-0.02	0.90	0.19	0.20
Cysteine	-0.02	0.91	0.08	0.58
Valine	-0.02	0.87	0.14	0.35
Methionine	-0.02	0.88	0.20	0.17
Isoleucine	-0.03	0.84	0.14	0.35
Leucine	-0.06	0.67	0.12	0.43
Phenylalanine	-0.16	0.29	0.01	0.96
Lysine	-0.17	0.24	-0.01	0.96
Histidine	-0.18	0.23	-0.06	0.69
Arginine	-0.26	0.07	-0.12	0.41
Tryptophan	0.14	0.33	0.40	0.01

Table 2.6. Standardized amino acid digestibility (%) for the two lowest pepsin digestibility meat and bone meal (MBM) samples determined by the precision-fed cecectomized rooster assay (PFR) and precision-fed chick assay (PFC)

	MBM Sample Number							
	1				2			
Amino Acid	PFR[1]	SEM	PFC[2]	SEM	PFR	SEM	PFC	SEM
Threonine	46.24[a]	0.88	31.00[b]	3.22	47.40	0.88	49.38	2.91
Cysteine	26.54	1.68	22.77	4.59	18.78[b]	1.98	46.04[a]	3.78
Valine	51.49[a]	1.10	38.95[b]	1.89	51.20	1.20	56.70	2.90
Methionine	53.16[a]	0.79	36.06[b]	1.18	53.83	1.15	56.00	3.41
Isoleucine	52.28[a]	1.28	38.22[a]	1.60	51.91	1.28	57.58	3.20
Leucine	50.43[a]	0.90	37.01[b]	1.84	51.34	1.04	54.96	2.86
Phenylalanine	55.98[a]	0.99	41.86[b]	1.75	55.76	0.84	57.96	2.69
Lysine	39.71	1.70	35.00	1.64	39.83[b]	1.25	55.24[a]	3.43
Histidine	47.44[a]	1.31	30.92[b]	2.11	47.41	1.37	51.13	3.18
Arginine	61.89[a]	0.87	49.73[b]	1.26	61.94	0.87	64.14	2.55
Tryptophan	71.28[a]	1.58	39.25[b]	3.91	76.61[a]	1.95	52.29[b]	3.02

[a,b] Means within a row within sample number with no common superscripts are significantly different ($P<0.05$).
[1]Mean of 4 roosters.
[2]Mean of 4 replicate pens of 4 chicks.

Table 2.7. Standardized amino acid digestibility (%) for the two highest pepsin digestibility meat and bone meal (MBM) samples determined by the precision-fed cecectomized rooster assay (PFR) and precision-fed chick assay (PFC)

	MBM Sample Number							
	15				16			
Amino Acid	PFR[1]	SEM	PFC[2]	SEM	PFR	SEM	PFC	SEM
Threonine	84.56	1.19	84.16	1.76	86.09[a]	1.36	79.82[b]	1.06
Cysteine	66.74	1.75	71.46	3.94	65.04	2.74	65.98	1.88
Valine	85.82	1.03	85.34	1.39	86.69[a]	1.03	81.46[b]	0.90
Methionine	89.64	0.71	89.18	0.69	90.58[a]	0.71	84.44[b]	1.07
Isoleucine	87.66	1.03	87.66	1.05	88.93[a]	0.83	83.49[b]	0.95
Leucine	88.14	0.90	87.51	1.05	88.31[a]	0.88	82.56[b]	0.96
Phenylalanine	87.92	0.87	87.28	1.03	88.14[a]	0.84	82.77[b]	0.99
Lysine	80.70[b]	1.76	87.29[a]	1.06	78.33	3.88	82.56	0.96
Histidine	81.43[b]	0.83	85.59[a]	1.14	81.58	2.06	79.70	0.98
Arginine	85.23	1.92	88.72	1.34	84.51	0.84	84.41	0.95
Tryptophan	93.67[a]	1.85	88.15[b]	0.91	94.74[a]	1.08	80.17[b]	1.12

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

[1]Mean of 4 roosters.

[2]Mean of 4 replicate pens of 4 chicks.

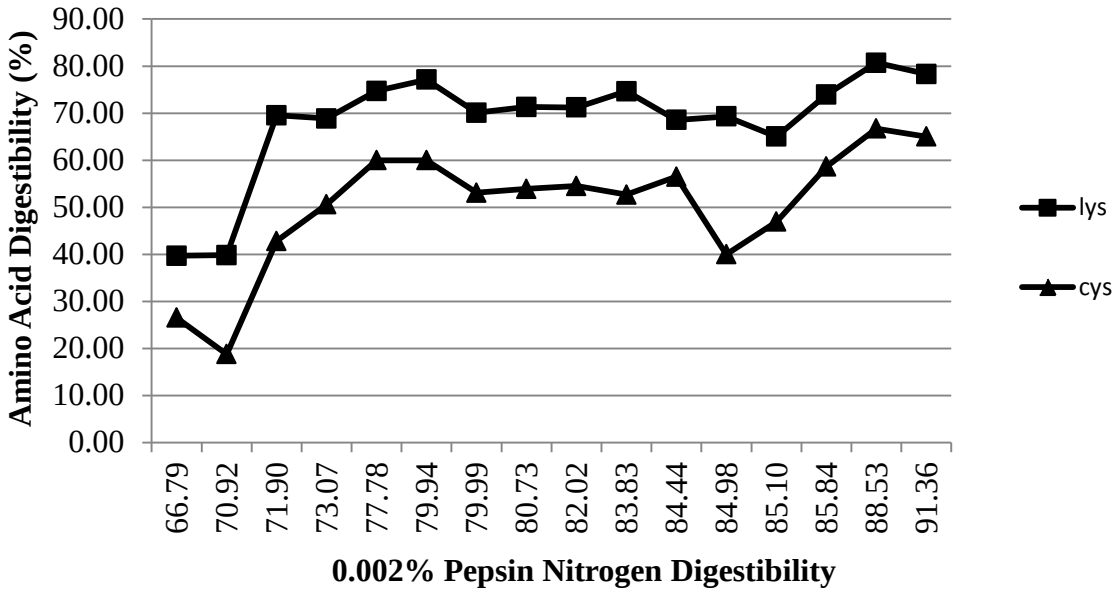

Figure 2.1. Plot of lysine and cysteine digestibility in roosters versus pepsin nitrogen digestibility for all 16 meat and bone meal samples. Correlation coefficients for lysine and cysteine were 0.70 and 0.64, respectively ($P<0.0001$).

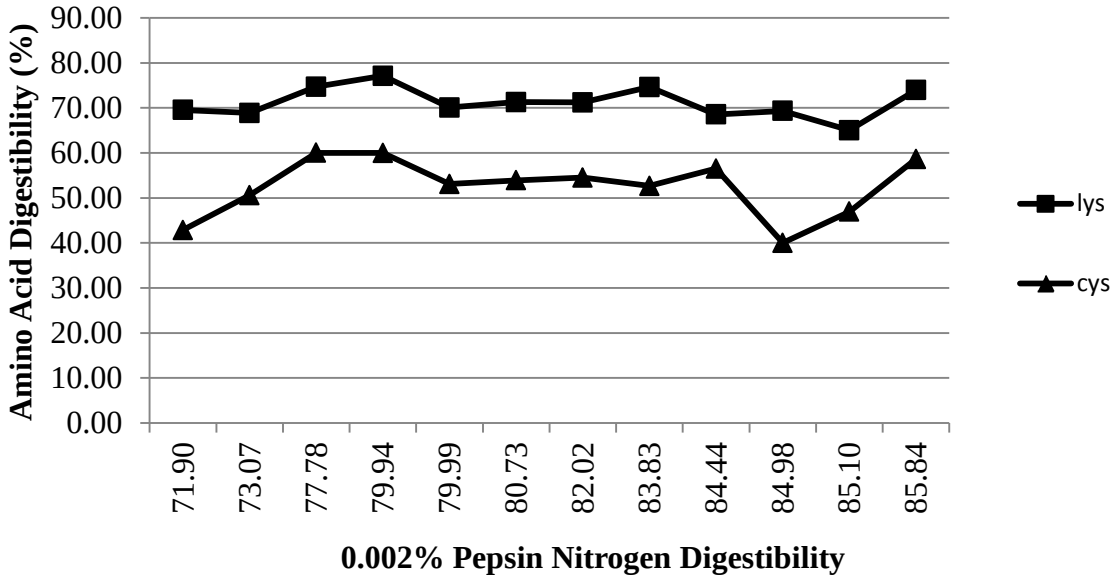

Figure 2.2. Plot of lysine and cysteine digestibility in roosters versus pepsin nitrogen digestibility excluding the two highest and lowest pepsin digestibility samples. Correlation coefficients for lysine and cysteine were -0.01 and 0.08, respectively, and were not significantly different from zero ($P<0.96$ and $P<0.58$, respectively).

Chapter 3

COMPARISON OF FOUR DIFFERENT ASSAYS TO DETECT DIFFERENCES IN AMINO ACID DIGESTIBILITY AMONG MEAT AND BONE MEALS

ABSTRACT

Four meat and bone meal (MBM) samples were selected and evaluated based on differences in pepsin nitrogen digestibility values (two higher pepsin digestibility samples and two lower pepsin digestibility samples). Pepsin nitrogen digestibility was determined using either 0.02 or 0.002% pepsin. Values ranged from 80.6 to 90.8% for 0.02% pepsin and from 73.2 to 87.3% for 0.002% pepsin. The two high and two low MBM samples averaged 90 and 82% digestibility in 0.02% pepsin, respectively, and the two high and two low MBM samples averaged 84 and 73% pepsin digestibility in 0.002% pepsin, respectively. The precision-fed cecectomized rooster assay and the standardized ileal amino acid (AA) digestibility chick assay were then used to determine standardized AA digestibility for the four MBM samples. Both assays yielded higher AA digestibility values for the two higher pepsin digestibility samples than for the two lower pepsin digestibility samples. The rooster assay generally yielded higher ($P<0.05$) AA digestibility values than the chick ileal assay. In addition, a slope-ratio chick growth assay was conducted to determine Lys bioavailability and this assay yielded values that were in general agreement with the rooster and chick ileal digestibility assays for two of the MBM but not for the two other samples. These results indicate that the pepsin, cecectomized rooster and chick ileal digestibility assays were in general agreement for detecting differences in AA digestibility among MBM samples but the chick growth assay was less consistent.

INTRODUCTION

Meat and bone meals are used in many poultry feeding programs. The quality of MBM can vary due to raw ingredients, processing conditions, and storage conditions (Johnston and

Coon, 1979a). Quality control methods, such as the pepsin digestibility assay (AOAC, 1980) can be very useful in determining the quality of MBM samples. As shown in Chapter 2, the pepsin digestibility assay is particularly useful for detecting low quality samples. Johnston and Coon (1979b) and Parsons et al. (1997) showed that reducing the pepsin concentration from 0.2% to 0.002% greatly increased the sensitivity of the assay for detecting differences in protein quality among MBM samples. Parsons et al. (1997) further reported that reducing the pepsin concentration from 0.002 to 0.0002% for MBM assays yielded little or no advantage.

Digestibility assays are the most common techniques used to estimate AA bioavailability. The precision-fed cecectomized rooster assay is a common method used to determine AA digestibility (Ravindran and Bryden, 1999; Parsons, 2002). Birds are fasted 24-48 hours prior to feeding a measured quantity of sample and then excreta are then quantitatively collected over a period of 48 hours and analyzed for amino acids. A major advantage of this assay is that many feed ingredients can be tested in a relatively short time with few birds and the roosters can be used for several assays (Lemme et al., 2004). The standardized ileal AA digestibility (SIAAD) chick assay is also a common method used to determine AA digestibility and involves feeding a test diet containing the test ingredient as the only source of dietary protein. Chicks are then humanely euthanized and digesta contents in the ileum are collected and analyzed for AA (Adedokun et al., 2007, 2008; Ravindran and Bryden, 1999). The slope-ratio chick growth assay is another assay that can be used to estimate AA bioavailability. Slope-ratio assays use diets that are formulated to be deficient in a specific AA and then increasing levels of the AA or test ingredient are added to produce linear increases in response criteria (Batterham et al., 1986). The common response criteria measured in this assay include body weight gain, gain:feed ratio, and nitrogen retention (Sibbald, 1987).

Little or no research has been conducted to evaluate and compare all four of the above assays in the same study. Therefore, the primary objective of the current study was to compare the pepsin nitrogen, precision-fed cecectomized rooster, chick ileal and slope-ratio chick growth assays for detecting differences in AA digestibility among MBM.

MATERIALS AND METHODS

Meat and Bone Meals

Four MBM samples were obtained from various commercial rendering plants from Darling International, Inc., Irving, Texas in attempt to obtain samples that varied in pepsin digestibility. The first two samples were obtained from commercial plants that had high pepsin nitrogen digestibility in Chapter 2 and Samples 3 and 4 were selected from plants that had low pepsin nitrogen digestibility in Chapter 2. Pepsin nitrogen digestibility was determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 1980) at Darling International Analytical Laboratory in Ankeny, Iowa except that the recommended level of 0.2% pepsin was reduced to 0.02 and 0.002% pepsin concentrations.

Precision-fed Cecectomized Rooster Assay

All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. Mature cecectomized Single Comb White Leghorn roosters were utilized in the precision-fed rooster assay according to the procedures of Parsons (1985). After 26 h without feed, four roosters were tube-fed 30 grams of a MBM sample. The roosters were then placed in a cage with a plastic tray underneath, and the total excreta were collected for 48 hours. The excreta samples were frozen and stored at -20˚C then freeze-dried, ground, and the MBM and dried excreta were analyzed for AA at the University of Missouri. Digestibility of amino acids was then calculated for each of the 20 roosters.

Standardized Ileal Amino Acid Chick Assay

The standardized ileal AA chick assay was conducted using the procedures described by (Adedokun et al., 2008). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. One-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery, weighed individually, wing-banded, and fed a nutritionally complete starter diet until d 16 before they were placed on the experimental diets. Six groups of 5 chicks were assigned to an experimental diet until 21 d of age. All diets were formulated to contain 20% CP solely provided by the MBM samples. Feed and water were supplied ad libitum. Birds were euthanized via CO_2 on d 21 and ileal digesta were collected from the Meckel's diverticulum to the ileal-cecal junction. Ileum contents were pooled, frozen, and stored at -20˚C then freeze-dried, ground by using a mortar and pestle, and analyzed for AA and chromium at the University of Missouri.

Slope-Ratio Chick Growth Assay

Bioavailability of Lys was determined in the slope-ratio chick growth assay by using a lysine-deficient corn-corn gluten meal-soybean meal diet (Table 3.1). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. Eight-day-old chicks resulting from the cross of New Hampshire males and Columbian Plymouth Rock females were fed a nutritionally complete starter diet during the first week posthatching. On d 8, chicks were weighed, wing-banded, and allotted to dietary treatments as described by Sasse and Baker (1973). Five groups of 5 chicks (3 females, 2 males) were assigned to each experimental diet. Feed and water were supplied ad libitum.

Two levels of crystalline test Lys (0.10% and 0.20%) from L-Lys·HCl were added to the Lys deficient basal diet to produce a standard growth curve. Each MBM sample was added to

the basal diet at the two Lys levels to provide 0.10% and 0.20% total Lys based on AA analysis of the MBMs. The L-Lys·HCl and the MBMs were added to the diets in place of cornstarch. The 11 diets were fed from 8 to 21 d of age.

Calculations

Standardized AA digestibility values for the precision-fed cecectomized rooster assay were calculated using the following formula. Amino acids were standardized using an endogenous correction based on AA excretion by fasted roosters.

Standardized AA digestibility, % = [(AA consumed, mg – AA excreted, mg + endogenous AA excreted, mg) / AA consumed, mg] x 100.

Standardized AA digestibility values for the SIAAD were calculated using the following formula by Moughan et al. (1992).

Apparent ileal AA digestibility = [1 – (chromium in diet/chromium in digesta) x (AA in digesta/AA in diet)] x 100,

Standardized AA digestibility, % = apparent digestibility + [(ileal endogenous AA flow, g/kg of DM intake)/(AA in the diet, g/kg of DM intake)] x 100.

Statistical Analysis

Data from the precision-fed cecectomized rooster, SIAAD, and slope-ratio chick growth assays were analyzed by ANOVA and PROC GLM tests (SAS Institute, 2008) for a completely randomized design. Statistical significance of differences among individual treatments was then determined using the least significant difference test (Carmer and Walker, 1985). Statistical significance was indicated at $P < 0.05$. For the chick growth assay data, a multiple linear regression of weight gain (Y) on supplemental Lys intake from L-Lys·HCl or a MBM (X) was then computed. Bioavailability of the Lys in the MBMs was calculated by dividing the

regression coefficient for the MBMs by that for L-Lys·HCl using the slope ratio method (Finney, 1978).

RESULTS AND DISCUSSION

The pepsin nitrogen digestibility values for the four meat and bone meal samples ranged from 80.6 to 90.8% for 0.02% pepsin (Table 3.2), and the values for the 0.002% pepsin nitrogen digestibility ranged from 73.2% to 87.3%. The pepsin digestibility values for Samples 1 and 2 were higher than those for Samples 3 and 4 at both pepsin concentrations. Thus, we were successful in obtaining MBM samples that varied substantially in pepsin nitrogen digestibility.

The total AA concentrations of the four MBM samples presented in Table 3.3 showed a substantial amount of variation among samples. Variation in raw material composition was likely the primary cause of variation in AA concentrations among samples (Parsons et al., 1997).

The standardized AA digestibility values for the four MBM samples determined by the precision-fed cecectomized rooster assay and SIAAD are presented in Table 3.4. For Samples 1 and 2, AA digestibility values were significantly higher for roosters than for chicks for several AA. For Samples 3 and 4, digestibility values for all AA were significantly higher for roosters than chicks. The higher AA digestibility values for the precision-fed cecectomized rooster assay than for SIAAD have been observed for some feed ingredients in earlier research conducted by Kim et al. (2012). As discussed by Kim et al. (2012), the lower values for the SIAAD assay may be largely due to collecting digesta from the entire ileum (Meckel's diverticulum to the ileal-cecal junction) which may result in collection of some AA that may have been digested and absorbed in the lower or posterior part of the ileum. Both assays were able to detect differences in AA digestibility among the MBM samples. Samples 1 and 2 exhibited higher AA digestibility values than Samples 3 and 4 in both the rooster and chick digestibility assays.

In the Lys bioavailability chick assay, both weight gain and gain to feed ratio increased linearly in response to supplemental crystalline Lys and MBM (Table 3.5). The Lys bioavailability values varied from 56% in MBM 4 to 85% in MBM 1. The high pepsin digestibility MBM Sample 1 had significantly higher Lys bioavailability than the two lower pepsin digestibility MBM Samples 3 and 4. In addition, the bioavailability of Lys in the high pepsin digestibility MBM Sample 2 was significantly higher than that in the lowest pepsin digestibility MBM Sample 4. In contrast, the Lys bioavailability value for the low pepsin digestibility MBM Sample 3 was not significantly different and was actually numerically higher than the value for the high pepsin digestibility MBM Sample 2.

All four of the assays were in general agreement regarding nitrogen and AA digestibility among the MBM samples. The nitrogen digestibility values based on the pepsin assay clearly showed higher pepsin digestibility values for Samples 1 and 2 compared to Samples 3 and 4. Standardized AA digestibility results based on the precision-fed cecectomized rooster assay and the SIAAD assay also indicated higher AA digestibility values for Samples 1 and 2 than for Samples 3 and 4. The Lys bioavailability chick assay also yielded a higher Lys bioavailability value for MBM Sample 1 compared to Sample 4.

Although the Lys bioavailability assay was in general agreement with the three other assays, but there were a few exceptions. The primary difference was for MBM Sample 2 which had a numerically lower Lys bioavailability value than Sample 3; the reverse was observed in the rooster and chick digestibility assays. When comparing the actual numerical values among assays, the Lys bioavailability value of 85% for Sample 1 was in good agreement with the rooster and chick digestibility values. For Sample 2, the Lys bioavailability value of 66% was lower than the rooster and chick Lys digestibility values. For Sample 3, the Lys bioavailability

value of 74% was in agreement with the rooster digestibility value but was higher than the chick digestibility value for 65%. For Sample 4, the Lys bioavailability value was lower than both the rooster and chick digestibility values. Thus, although the Lys bioavailability assay was in general agreement with the rooster and chick digestibility assays, there were some inconsistencies.

In conclusion, the results from the current study indicate that the pepsin, cecectomized rooster and chick ileal digestibility assays are all useful methods to detect differences in AA digestibility among MBM samples. The Lys bioavailability chick growth assay was also a useful assay but was less consistent.

LITERATURE CITED

Adedokun, S.A., C.M. Parsons, M.S. Lilburn, O. Adeola, and T.J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590-2597.

Adedokun, S.A., O. Adeola, C.M. Parsons, M.S. Lilburn, and T.J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535-2548.

Association of Official Analytical Chemists. 1980. Official Methods of Analysis. 13th Rev. ed. Association of Official Analytical Chemists, Washington, D.C.

Batterham, E.S., R.E. Darnell, L.S. Herbert, and E.J. Major. 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal as determined by slope-ratio assays with growing pigs, rats and chicks and by chemical techniques. Br. J. Nutr. 55:441-453.

Carmer, S.G., and W.M. Walker. 1985. Pairwise multiple comparisons of treatment means in agronomic research. J. Agron. Educ. 14:19-26.

Finney, D.J. 1978. Statistical Method of Biological Assay. 3rd ed. Charles Griffen and Co. Ltd., High Wycombe, Buckinghamshire, UK.

Johnston, J., and C.N. Coon. 1979a. A comparison of six protein quality assays using commercially available protein meals. Poult. Sci. 58:919-927.

Johnston, J., and C.N. Coon. 1979b. The use of varying levels of pepsin for pepsin digestion studies with animal proteins. Poult. Sci. 58:1271-1273.

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91:1350-1355.

Lemme, A., V. Ravindran and W.L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World Poult. Sci. J. 60:423-437.

Moughan, P.J., G. Schuttetr, and M. Leenaars. 1992. Endogenous amino acid flow in the stomach and small intestine of the young growing pig. J. Sci. Food Agric. 60:437-442.

Parsons, C.M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers dried grains with solubles. J. Agric. Sci. 104:469-472.

Parsons, C.M. 2002. Digestibility and bioavailability of protein and amino acids. pp 115-135 In: Poultry feedstuffs: Supply, composition, and nutritive value J.M. McNab and K.N. Boorman (ed.). CABI, Oxon, UK.

Parsons, C.M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poult. Sci. 76:361-368.

Ravindran, V., and W.L. Bryden. 1999. Amino acid availability in poultry – in vitro and in vivo measurements. Aust. J. Agric. Res. 50:889-908.

SAS Institute. 2008. SAS User's Guide. Statistics. Version 9.2 ed. SAS Inst. Inc., Cary, NC.

Sasse, C.E., and D.H. Baker. 1973. Availability of sulfur amino acids in corn and corn gluten meal for growing chicks. J. Anim. Sci. 37:1351-1355.

Sibbald, I.R. 1987. Estimation of bioavailable amino acids in feedingstuffs for poultry and pigs: A review with emphasis on balance experiments. Can. J. Anim. Sci. 67:221-300.

TABLES

Table 3.1. Composition of the lysine-deficient basal diet used in the slope-ratio chick growth assay

Ingredient	Amount (%)
Corn	29.88
Corn Gluten Meal	25.00
Corn Starch	30.00
Soybean Meal	8.00
Soybean Oil	2.00
Dical	2.25
Limestone	1.25
Vitamin premix[1]	0.20
Mineral premix[2]	0.15
Sodium Chloride	0.40
Choline Chloride	0.20
L-Tryptophan	0.03
L-Threonine	0.07
L-Arginine	0.40
DL-Methionine	0.04
Bacitracin	0.04

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

[2]Provided as milligrams per kilogram of diet: manganese, 75 (from $MnSO_4 \cdot H_20$); iron, 75 (from $FeSO_4 \cdot H_20$); zinc, 75 (from ZnO); copper, 5 (from $CuSO_4 \cdot 5H_20$); iodine, 0.75 (from ethylene diamine dihydroiodide); selenium, 0.1 (from Na_2SeO_3).

Table 3.2. Pepsin nitrogen digestibility values for the four meat and bone meal samples[1]

Sample Number	0.02% Pepsin	0.002% Pepsin
1	90.8	87.3
2	89.9	81.3
3	82.6	73.8
4	80.6	73.2

[1]Values are means of triplicate analyses.

Table 3.3. Total amino acid concentrations (%) of the four meat and bone meal (MBM) samples, as-fed basis

	MBM Sample Number			
Amino Acid	1	2	3	4
Aspartic Acid	3.86	4.43	3.66	4.10
Threonine	1.77	1.95	1.67	1.83
Serine	1.79	1.80	1.71	1.63
Glutamic Acid	6.13	7.16	5.83	6.41
Proline	3.60	4.00	3.70	3.58
Alanine	3.57	4.02	3.51	3.78
Cysteine	0.49	0.52	0.46	0.40
Valine	2.39	2.75	2.23	2.65
Methionine	0.78	0.87	0.62	0.82
Isoleucine	1.72	1.99	1.54	1.85
Leucine	3.43	3.92	3.27	3.80
Tyrosine	1.49	1.74	1.39	1.63
Phenylalanine	1.90	2.18	1.83	2.17
Lysine	3.07	3.52	2.68	3.23
Histidine	1.13	1.53	1.06	1.40
Arginine	3.37	3.80	3.39	3.44
Tryptophan	0.45	0.48	0.37	0.39

Table 3.4. Standardized amino acid digestibility (%) for the four meat and bone samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility chick assay (SIAAD)

	MBM Sample Number							
	1				2			
Amino Acid	PFR[1]	SEM	SIAAD[2]	SEM	PFR	SEM	SIAAD	SEM
Aspartic Acid	84.70[a]	1.22	80.71[b]	0.69	71.80	1.85	66.92	2.03
Threonine	87.69[a]	1.06	84.30[b]	0.77	80.94	2.18	74.44	2.24
Serine	83.13	1.56	80.14	0.97	75.81	2.88	72.29	2.56
Glutamic Acid	87.90	0.93	87.47	0.54	81.81	1.49	78.47	1.61
Proline	82.23	1.71	84.07	0.58	78.89	1.77	80.53	0.73
Alanine	86.54	1.24	88.68	0.55	82.75	1.26	83.29	1.03
Cysteine	72.82[a]	1.12	57.29[b]	2.16	66.68[a]	4.48	52.64[b]	2.71
Valine	87.34[a]	0.86	84.42[b]	0.78	83.00	1.51	79.43	1.69
Methionine	91.76[a]	0.49	89.66[b]	0.51	86.67[a]	1.29	80.71[b]	1.89
Isoleucine	88.21[a]	0.73	84.61[b]	0.70	84.07[a]	1.32	78.07[b]	1.82
Leucine	89.26	0.79	87.40	0.66	85.00[a]	1.28	79.66[b]	1.59
Tyrosine	86.79[a]	0.65	84.19[b]	0.67	82.21[a]	1.67	76.19[b]	1.93
Phenylalanine	87.54	0.74	86.70	0.69	84.01	1.32	79.73	1.49
Lysine	86.53	0.76	88.52	0.55	79.10	1.77	78.67	1.86
Histidine	85.07	1.24	87.86	0.47	78.72	1.58	78.63	1.39
Arginine	88.22	1.35	89.47	0.62	87.98[a]	0.95	82.84[b]	1.42
Tryptophan	96.50[a]	0.49	92.56[b]	0.67	92.37[a]	0.84	82.51[b]	1.67

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

[1] Mean of 5 roosters.

[2] Mean of 6 replicate pens of 5 chicks.

Table 3.4 continued. Standardized amino acid digestibility (%) for the four meat and bone samples determined by the precision-fed cecectomized rooster assay (PFR) and standardized ileal amino acid digestibility chick assay (SIAAD)

	MBM Sample Number							
	3				4			
Amino Acid	PFR[1]	SEM	SIAAD[2]	SEM	PFR	SEM	SIAAD	SEM
Aspartic Acid	64.44[a]	1.23	47.79[b]	1.73	67.26[a]	1.40	55.67[b]	1.08
Threonine	76.46[a]	1.46	57.86[b]	2.03	76.10[a]	1.08	62.65[b]	0.81
Serine	73.05[a]	1.65	55.06[b]	1.92	73.79[a]	1.07	61.83[b]	1.33
Glutamic Acid	79.97[a]	1.08	63.48[b]	1.77	76.65[a]	0.64	66.35[b]	0.81
Proline	75.34[a]	1.27	69.19[b]	1.07	78.43[a]	1.31	73.93[b]	1.00
Alanine	78.47[a]	1.06	72.84[b]	1.23	77.95[a]	0.75	71.09[b]	0.83
Cysteine	62.34[a]	2.69	15.38[b]	3.94	64.58[a]	2.08	49.16[b]	1.72
Valine	78.35[a]	1.47	64.95[b]	2.23	77.29[a]	0.51	64.42[b]	0.75
Methionine	81.25[a]	1.73	63.33[b]	2.25	78.70[a]	0.60	60.85[b]	0.96
Isoleucine	78.94[a]	1.39	61.04[b]	2.62	77.78[a]	0.44	63.22[b]	0.74
Leucine	80.77[a]	1.29	65.02[b]	2.07	77.69[a]	0.62	64.71[b]	0.77
Tyrosine	78.44[a]	1.45	57.47[b]	2.35	77.39[a]	0.85	62.03[b]	0.83
Phenylalanine	79.93[a]	1.32	66.93[b]	1.88	77.53[a]	0.68	65.31[b]	0.74
Lysine	73.68[a]	1.92	64.58[b]	2.08	72.92[a]	1.02	64.19[b]	0.68
Histidine	75.99[a]	1.71	64.97[b]	1.92	74.57[a]	1.01	63.89[b]	0.63
Arginine	83.86[a]	1.02	71.45[b]	1.78	82.81[a]	0.74	72.12[b]	0.68
Tryptophan	90.73[a]	0.31	73.01[b]	1.39	90.97[a]	1.32	75.15[b]	0.71

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

[1] Mean of 5 roosters.

[2] Mean of 6 replicate pens of 5 chicks.

Table 3.5. Determination of Lys bioavailability in the four meat and bone meals (MBM) using a slope-ratio chick growth assay[1]

Treatment[2]	Weight Gain	Gain:Feed	Feed Intake	Supplemental Lys Intake	Lys Bioavailability[3]
	(g)	(g:kg)	(g)	(g)	(%)
1. Basal diet	105.4	346	306	0	
2. As 1 + 0.10% Lys	139.0	432	324	.324	
3. As 1 + 0.20% Lys	203.1	539	378	.756	100[a]
4. As 1 + 3.30% MBM1	130.0	434	300	.300	
5. As 1 + 6.50% MBM1	185.3	505	367	.734	84.8[b]
6. As 1 + 2.80% MBM2	132.4	427	317	.317	
7. As 1 + 5.70% MBM2	165.3	450	369	.738	66.5[c]
8. As 1 + 3.70% MBM3	133.6	431	310	.310	
9. As 1 + 7.50% MBM3	165.5	491	337	.675	74.0[bc]
10. As 1 + 3.10% MBM4	128.6	391	331	.331	
11. As 1 + 6.20% MBM4	149.9	443	341	.682	55.7[d]
Pooled SEM	5.6	36.5	15.8	.02	

[a-d]Values within the column with no common superscripts are significantly different ($P<0.05$).

[1]Means of five replicate groups of five chicks each from 8 to 21 d posthatching. Average initial weight was 99.4 g.

[2]The MBM were added at levels to provide 0.10 and 0.20% Lys based on amino acid analysis of the MBM samples.

[3]Calculated by the slope-ratio procedure. The multiple regression of gain on supplemental Lys intake from the different sources was: Weight gain = 101.91 + 130.9 ± 7.5 Lys + 111.0 ± 7.8 MBM1 + 87 ± 7.7 MBM2 + 96.8 ± 8.3 MBM3 + 72.9 ± 8.2 MBM4; $R^2 = 0.88$.