Project Title: **Digestibility of amino acids by Broilers and Turkeys**

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INDUSTRY SUMMARY

The primary objectives of this research was to a) quantify the endogenous losses of amino acids in broilers and turkey poults during the first three weeks of age, in order to establish a baseline protocol for correction of ileal digestible amino acids to a true digestible basis, and to b) determine the apparent and true digestibility from predominate feed ingredients in broilers and turkey poults with a new consensus protocol across nutrition laboratories. The long-term goal of our research is provide practical means to accomplish reductions in protein and amino acid formulation with adoption of the digestible amino acid concept. This research was a result of a digestible amino acid workshop for the broiler industry which was held February, 2004 in Indianapolis for academic researchers, all U.S. based amino acid suppliers, and formulating industry nutritionists. Since then, two additional annual meetings have been held and expanded across companies and inclusion of turkey nutritionists. Goals of the group are to fill gaps in knowledge and provide a publicly accessible database of information such that the industry is more able to adopt the digestible amino acid concept.

Short and long-range benefits - With impending shortages of corn and SBM, formulation of diets on a digestible amino acid basis is imperative to reduce feed cost. Further benefits of formulating on a digestible amino acid basis include decreasing safety margins, increasing the accuracy of predicting performance, and increasing the uniformity of product after processing. Source reduction technologies, such as dietary formulation reductions in crude protein, for the industry to lessen nitrogen emissions are imperative with pending ammonia emissions regulations in 2009.

Notably, the ileal amino acid digestibility bio-assay was validated across laboratories with no distinguishable differences. For standardization purposes, the regression of casein versus a nitrogen-free diet gave similar results. Either a 10% casein diet or nitrogen-free diet would be recommended for future standardization studies, as would feeding of a dietary crude protein content of 20 percent. Somewhat surprisingly, the endogenous loss associated during the first week after placement (5 days of age) was dramatically higher than 15 or 21 days of age (the later ages were not different from themselves), with the turkey being generally higher than that of the chick. Therefore for primary ingredient determinations, 21 days of age would be recommended. Given our estimations of endogenous losses during the first week, the endogenous loss contributes up to 2/3rds of the indigestibility versus older birds. Previously, the inefficient utilization of amino acids by young birds was mostly attributed to inadequate numbers of amino acid and peptide transporters. Further knowledge of how this endogenous loss could be minimized would be of great interest in improving early nutritional status of the hatchling.

Keywords: Broiler, Digestible amino acid, Endogenous loss, Turkey

Scientific Report:
Please see attached manuscripts:

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2006 Comparison of endogenous ileal amino acid and nitrogen flow in turkey poults and broiler chicks. *Poult. Sci.* ARP Number: 2006-17946 (manuscript submitted).

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. *Poult. Sci.* ARP Number: 2007-18074 (manuscript submitted).

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2007. Effect of Age, Method, and Location on Ileal Endogenous Amino Acid and Total Amino Acid Flows in Turkey Poults. *Poult. Sci.* (manuscript submitted).

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2007. Standardized Ileal Amino Acid Digestibility of Meat and Bone Meal in Broiler Chicks and Turkey Poults using a Nitrogen-free or Casein diet. *Poult. Sci.* (manuscript – internal review).

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2007. Standardized ileal amino acid digestibility of some feed ingredients of plant sources in broiler chicks and turkey poults using a nitrogen-free or casein diet. *Brit. Poult. Sci.* (manuscript – internal review).

The manuscripts are not intended for distribution, as the copyright rights have been transferred to each respective journal. Reprint copies will be made available from Dr. Applegate by request after publication.

List of Presentations & Publications

Applegate, T.J., W. Powers, and R. Angel. 2005. Feeding to reduce emissions from manure from non-ruminants. Minnesota Nutrition Conference Proceedings. pp 95-104.

Applegate, T.J., W. Powers, and R. Angel. 2006. Air emissions from turkey barns: regulations, science, and the philosophy of reductionism. Midwest Poultry Federation Proceedings. 16 pgs.

Applegate, T.J. 2006. Protein and amino acid nutrition in poultry: impacts on performance and the environment. Meat Quality and Feed Efficiency Conference. Dunboyne, Ireland.

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2006 Comparison of endogenous ileal amino acid and nitrogen flow in turkey poults and broiler chicks. Poult. Sci. ARP Number: 2006-17946 (manuscript submitted).

Adedokun, S.A., C. Parsons, M. Lilburn, O. Adeola, T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. J. Nutr. ARP Number: 2007-18074 (manuscript submitted).

Adedokun, S.A., C. Parsons, M. Lilburn, O.Adeola, and T. J. Applegate. 2006. Standardized ileal amino acid digestibility of meat and bone meal in broiler chicks using a nitrogen-free or casein diet. Poult. Sci. 85(Suppl. 1):86.

Adedokun, S.A., C. Parsons, M. Lilburn, O.Adeola, and T. J. Applegate. 2006. Comparison of endogenous ileal amino acid and total nitrogen flow in turkey poults and broiler chicks. Poult. Sci. 85(Suppl. 1):85.

Adedokun, S.A., C. Parsons, M. Lilburn, O.Adeola, and T. J. Applegate. 2007. Standardized ileal amino acid digestibility of plant source ingredients in broiler chicks and turkey poults using a nitrogen-free or casein diet. Poultry Sci. 85(Suppl. 1): in press.

Also a symposium is currently being organized for the joint ADSA/ASAS/PSA meeting in San Antonio, TX (July 2006), where a number of the results of this grant will be presented.

Section: Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interaction/Nutritional Methodologies and mathematical modeling

Effect of Age, Method, and Experimental Location on Ileal Endogenous Amino Acid and Total Amino Acid Flow in Broiler Chicks[1,2]

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Abstract

Determination of the contribution of ileal endogenous amino acid (IEAA) and total amino acid (TAA) to ileal digesta is important in determining standardized amino acid digestibility coefficient. The IEAA and TAA flow in broiler chicks at three ages (5, 15, and 21 d) and two experimental locations were determined using three different methods. The methods used were nitrogen-free diet (NFD), feeding of a completely digestible protein, casein, (CDP), and the regression (obtained by regressing dietary casein concentration against IEAA or TAA flow). Semi-purified diets containing 0 (NFD), 50, 100, or 150 g casein per kg diets with casein as the sole amino acids source were used. Each diet was fed for 5 consecutive days before birds were euthanized and ileal contents collected from six replicate cages of 30, 10, or 8 birds per cage on d 5, 15, or 21, respectively. Day 5 IEAA and TAA flow (mg/kg DMI) in chicks fed NFD were higher ($P < 0.05$) (methionine=154, threonine=539) than values for d 15 (methionine=51, threonine=274) and d 21 (methionine=50, threonine=274) which were not significantly different. There was no significant interaction between location and diet on d 5 with an increase ($P < 0.05$) in IEAA and TAA flow from NFD (methionine=154, threonine=538.6) to diet containing 150 g casein (methionine=392, threonine=1,148). Significant interaction ($P < 0.05$) between location and diet was obtained for most of the amino acids and TAA for d 15 and d 21. The trend for IEAA and TAA flow on d 15 and 21 were similar in both locations with an increase ($P < 0.05$) from 0 g to 150 g casein/kg diet. Estimates of IEAA and TAA flow using the regression method for methionine and threonine were 216, and 657 (location 1 & 2) on d 5. Comparison between the regression and the NFD methods showed a higher flow on d 5 for the NFD method. There was no difference between the two methods on d 15 and 21 except for lysine, methionine, and glutamic acid (d 21). The results obtained from this study indicate the presence of interaction between location and diet and location and age. Also the results indicate that as birds aged, a decrease in IEAA and TAA flow occurred which became stable between d 15 and 21. Comparison between the NFD and the regression method showed that both methods will give similar results on d 15 and 21.

(Word count: 398 instead of 250 words)

Key words: Casein, chick, endogenous amino acid, nitrogen-free diet, regression

Introduction

In an attempt to improve nutrients utilization in chicks it is important to accurately determine nutrient digestibility. Part of the efforts aimed at reducing errors associated with nutrient digestibility is the use of ileal rather than total tract digestibility values. By using ileal digestibility values, errors associated with the excretion of the by products of nutrient metabolism (e.g. urine nitrogen) into the terminal portion of the gastro-intestinal tracts is removed as well as the effects of microbial fermentation in the hind gut **(1).** Apparent ileal digestibility, which is widely accepted and used, does not accurately reflect the true digestibility values of various feed ingredients because it does not account for basal and diet specific endogenous amino acid flow. For instance, it has been reported that basal endogenous nutrient (BEN) flow, as it is the case with amino acids and N, contribute to apparent digestibility values. The BEN arises from various sources which range from the salivary and various digestive secretions (enzymes), sloughed epithelial cells, and intestinal microbes **(2)**. In addition to this, up to 25% of daily protein synthesis has been reported to be secreted into the gastro-intestinal tract

(3). In addition to the BEN contribution to apparent digestibility values, diets specific endogenous contribution has been identified. Although, the latter is difficult to measure the former (BEN) has been measured using different methods **(1)**. Values obtained from the BEN flow can be used to correct the apparent digestibility coefficient of amino acids to obtain standardized ileal digestibility coefficients.

Basal ileal endogenous amino acids (IEAA) and TAA flow has been estimated in chickens of various ages (6, 15, 21, 28, 80 wk), using different techniques **(4)**, and in different classes of chickens - broiler, layer, and roosters **(5)** with significant effects of age on IEAA flow in most cases. Methods used varied from feeding of nitrogen free diet, NFD, (6), feeding of completely digestible protein (CDP), homoarginine method using guanidination reaction, feeding of enzyme hydrolyzed casein (7), and regression method. Based on the fact that nutrient composition of the diet for much of the first 3 wk of age in broilers is the same, it is important to evaluate the effects of age and the method of estimation on the quantity of IEAA and TAA flow in the terminal ileum. Also, it is important to estimate the effects of how IEAA flow in the chick may vary depending on site specific conditions of the experiment.

In order to evaluate this, we estimated IEAA and TAA flow in chicks at different ages (d 5, 15, and 21) using three different methods (feeding of NFD, CDP, and the use of the regression method). The same study was replicated in another location to determine the possibility of age by experimental location or diet by location effect. Our hypothesis was that IEAA and TAA flow are not age dependent and that they are location independent.

Material and Methods

Diet formulation. Four semi-purified diets were formulated to contain graded levels of casein. The protein contents of the diets were supplied entirely by the casein that was added at 0, 50, 100, or 150 g/kg of the diet. The dietary composition of the experimental diets and the analyzed values of the different amino acids and TAA are reported in Tables 1 and 2, respectively. A positive control diet that meets or exceeds the NRC (1994) recommendations was also made. This is the diet on which chicks were before they were placed on the four semi-purified diets. For IEAA and TAA flow calculations, chromic oxide was added to the treatment diets at 3 g/kg diet as an indigestible marker. All the diets were formulated and made at a single location and were from the same batch.

Birds, housing, and feeding. One thousand one hundred and fifty two 1-d old male broiler chicks (Ross 308, Aviagen, Huntsville, AL) each were used in each of the two experimental locations. Chicks were obtained from commercial hatcheries. Seven hundred and twenty birds in each experimental location were weighed individually and randomly allocated to diets on d 0. Each diet was fed for 5 d before ileal contents collection on d 5, 15, and 21. Six replicate cages containing 30 chicks per cage were euthanized and the ileal contents removed on d 5. The remaining chicks were fed a conventional corn and soybean meal-based starter diet until day 10 when 240 chicks were randomized to cages with 10 birds per cage and 6 replicate cages per diet. Birds were euthanized and the ileal contents collected on day 15. On day 16, 192 birds that have been on the positive control diet were placed on the experimental diets and were euthanized and ileal contents collected on day 21. All euthanasia was by CO_2 asphyxiation.

For the entire period of the study, chicks were raised in battery cages (Alternative Design Manufacturing and Supply, Inc. Siloam Springs, AR) in an environmentally controlled room with 24-h of light. Room temperature for the first, second, and third week were 35, 30, and 25 ^{o}C, respectively. Feed and water were provided ad libitum. All animal care procedures were approved by the Purdue University and University of Illinois, Urbana/Champaign Animal Care and Use Committee.

Sampling and ileal digesta processing. On d 5, 15, and 21 after the birds had been euthanized by CO_2 asphyxiation, content from the ileal (the portion of the small intestine from Meckel's diverticulum to about 5 mm proximal to the ileo-cecal junction) region was flushed with distilled water. For birds sampled on d 5, 50 ml syringe was used for flushing while wash bottle was used on d 15 and 21. Ileal digesta from birds within a cage was pooled, frozen and stored at -40 ^{o}C until they were processed. Samples were freeze-dried, ground using mortar and pestle, and were sent to the University of Missouri Experiment Station and Chemical Laboratory for complete amino acid profile and chromium analysis.

Chemical analysis. Dry matter content was determined on ground diets and ileal digesta by drying the samples at 100^{o}C for 24 h. Amino acids and chromium analyses were conducted at the University of Missouri Experiment Station Chemical Laboratory. For amino acid analyses, samples were hydrolyzed in a 6 *N* HCl for 24 h at 110^{o}C under N atmosphere. Sulfur containing amino acids (methionine and cysteine) was determined by acid hydrolysis after performic acid oxidation. For tryptophan analysis, samples were hydrolyzed using barium hydroxide. The amino acids in the hydrolysate were then determined by HPLC after postcolumn derivatization (AOAC, 2000; 982.30 E [a, b, c]. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Chromium was determined by the inductively coupled plasma atomic emission spectroscopy method (AOAC, 2000; 990.08) following nitric/perchloric acids wet ash digestion.

Calculations. Ileal endogenous amino acid and TAA flow was calculated as mg of amino acid or TAA flow per kg of dry matter intake (DMI) using the formula proposed by **(8)**:

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

Comparison of the IEAA and TAA flows between the NFD and the regression method was made by calculating the standard errors of difference of means as outlined in Samuels and Witmer (1999). The probabilities of the t-values were determined using the t-test of SAS

Statistical analysis. Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Orthogonal polynomial contrasts (linear) were used to compare the treatment means. Differences between treatment means were separated using Duncan multiple range test where F-ratios indicated significance. The level of significance was set at $P < 0.05$.

Results

Birds in the two locations were in good condition of health throughout the duration of the study. Mortality (from location 1) on d 5 was 0.5% for each of the 4 diets, 0% for birds sampled on d 15 and on d 21 it was 2.1% for NFD and diet containing 10% casein. The mortality in location 2 was 0.5% (100 g casein diet) on d 5 and 1.7% (150 g casein diet) on d 15. Five day average feed intake/bird (mean±SEM) for birds sampled on d 5, 15, and 21 were, respectively 29±0.8, 119±3.6, 163±5.3 g for NFD, 29±0.8, 114±3.9, and 147±5.3 g for diet containing 50g casein/kg diet, 29±0.8, 115±3.6, 166±5.8 g for diet containing 100 g casein/kg diet, and 34±0.8, 132±3.6, 193±5.3 g for diet containing 150 g casein/kg diet. Mean body weight gain of birds on the NFD were 2±0.5, -6±1.6, and -19±4.4 g for d 5, 15, and 21, respectively. When the diet containing 50 g casein was fed, mean weight gain was 8±0.5, 6±1.7, and -8±4 g for birds sampled on d 5, 15, and 21, respectively. The corresponding body weight gain for birds fed diets containing 100 or 150 g casein/kg diet were 13±0.5, 18±1.6, and 22±4.8 or 18±0.5, 39±1.6, and 39±4.4 g, respectively for birds sampled on d 5, 15, and 21.

The IEAA flow in chicks fed NFD is reported in Table 3. There were no significant interactions between treatment and age when NFD was fed hence data from the two locations were pooled. Ileal EAA flow on d 5 is higher $(P < 0.05)$ than on d 15 and 21. There was no significant difference in IEAA and TAA flow between d 15 and 21. Amino acids with the greatest flow were glutamic acid (d 5=1,000, d 21=420 mg/kg DMI), aspartic acid (d 5=799, d 21=340 mg/kg DMI), leucine (d 5=633, d 21=251 mg/kg DMI) and threonine (d 5=539, d 21=274 mg/kg DMI). The IEAA flow on d 21 for methionine, threonine, and TAA on d 21 was, respectively, 32, 51, and 46% of their respective flow on d 5.

The effect of age of broiler chicks on IEAA flow when 50, 100, or 150 g casein/kg diet was fed is reported in Tables 4, 5, and 6, respectively. As the birds progressed in age, IEAA flow decreased $(P < 0.05)$ from d 5 to d 21. However, there was no difference in flow between d 15 and 21 at all levels of casein except for 150 g casein diet where flow on d 21 was higher $(P < 0.05)$ than on d 15. Significant interactions between location and age were observed for most of the amino acids and TAA.

Ileal endogenous amino acids and TAA flow in the terminal ileum of broiler chicks on d 5, 15, and 21 when fed diets containing graded levels of casein are reported in Table 7, 8, and 9. There was no location by diet interaction for all the amino acids and TAA on d 5 (Table 7). A significant interaction between location and diet was observed on d 15 and 21 when diets containing four levels of casein were fed (Tables 8 and 9). Ileal EAA flow increased linearly ($P < 0.05$) with increasing levels of casein in the diets at all ages studied. When 0 (NFD), 50, or 100 g casein/kg diet were fed (d 5), ileal endogenous methionine, threonine, and TAA was 39, 50, and 41% or 70, 73, and 71% or 84, 82, and 82%, respectively, of methionine, threonine, and TAA flow when 150 g casein/kg diet was fed.

On d 15 (Table 8), there were interactions ($P < 0.05$) between location and diet for some of the amino acids (histidine, isoleucine, lysine, methionine, threonine, alanine, aspartic acid, glutamic acid, praline, serine, and TAA). In location 1, the trend was an increase ($P < 0.05$) in IEAA flow from 0 to 150 g casein/kg diet with mean IEAA and TAA flow between 50 and 100 g casein diet not significantly different (data not shown). However, for location 2 (data not shown), the overall trend was similar to that of location 1, except that there was no significant difference in mean values of IEAA and TAA flow for birds on diet containing 100 and 150 g casein/ kg diet. At both locations, IEAA and TAA flow increased ($P < 0.05$) linearly with age.

Significant interaction between location and diet was obtained on d 21 except for arginine, leucine, lysine, methionine, phenylalanine, cysteine, and tyrosine (Table 9). Ileal EAA and TAA flow showed similar trends at both locations (data not shown) with linear ($P < 0.05$) increase from 0 to 150 g casein/kg diet. Ileal EAA and TAA flow using the regression method is reported in Table 10. Methionine, threonine, and TAA flow were 33, 50, and 37% (d 15) or 14, 30, and 27% (d 21) that of d 5. Comparison of endogenous amino acid and TAA flows between the NFD and the regression methods showed significant difference (d 5) between the two methods with higher *($P < 0.05$)* IEAA and TAA flows from the regression method. However, IEAA and TAA flows on d 15 and 21 were not different except for lysine, methionine, and glutamic acids where flows from the NFD method was higher (d 21) (Table 10).

Discussion

The objective of this study was to evaluate the effects of age of broiler chicks on IEAA flow and methods of estimation on IEAA and TAA flow in the terminal ileum of broiler chicks at d 5, 15, and 21. This study was also designed to test for location by age and location by diet interactions. In order to optimize the usage of dietary nutrient and to reduce nutrient excretion, it is important to determine the proportion of the amino acid in the diet that is digested and absorbed. To achieve this objective the contribution of endogenous secretion to ileal amino acid and TAA flow has to be determined. This will enable nutritionists to be able to formulate diets that closely meet bird's requirements which at the same time will reduce nutrient excretion. The determination of IEAA flow will allow us to be able to determine the standardized ileal amino acid digestibility (SIAAD) values of feed ingredients by correcting for basal endogenous amino acid loss. The other major objective was to compare results from two different locations to observe how repeatable and reliable results from different laboratories can be. To reduce variations in diet composition, all diets were made from the same batch of ingredients, mixed at one time and at one location, and all the chemical analyses were conducted at the same laboratory using the same protocol, albeit at slightly different times.

A number of studies have evaluated IEAA flow in chickens using different approaches, in different classes of chickens, and at different ages **(5)**. The uniqueness of our study is that IEAA flow was determined at relatively younger ages (d 5, 15, and 21) using three different methods (NFD, feeding of CDP, and the regression methods). It has been reported that each of these methods is based on certain assumptions with its peculiar limitations **(2)**, we hope that being able to present data from the same set of birds and ages using three different methods under the same experimental setting will enable us to be able to compare these results based on the different assumptions on which they are based.

Ileal endogenous amino acids and TAA flow was highest on d 5 when determination was by NFD, CDP, or the regression method. However, flow on d 15 and 21 was considerably low and there was no significant difference between flows at these ages (d 15 and d 21) except when diet containing 150 g casein was fed. On d 5, IEAA and TAA flow was about twice (150 g casein diet) or more than double (NFD, 50, or 100 g casein diets) the values for d 15 or d 21. The amino acids with the greatest flow were glutamic acid, aspartic acid, leucine, threonine, proline, and serine. At all the ages, the flow of methionine and histidine were the least (methionine<histidine). This could be attributed to the fact that these amino acids, especially methionine are rapidly and almost completely absorbed in the gastro-intestinal tract. The high level of glutamic acid and aspartic acid in the flow could be as a result of the importance of these amino acids especially, glutamic acid in the gastro intestinal tract metabolism. Because of the nature of the diet (NFD, CDP), the sources of endogenous secretion could either be from mucoproteins, sloughed cells, or from the various digestive secretions (enzymes). These results show that at younger age (d 5) the contribution to amino acids of endogenous origin to ileal digesta was higher (about two times greater) relative to d 15 and d 21. These endogenous secretions however remain stable between d 15 and 21. Going by the amino acid with the highest flow, it can be argued that when NFD is fed, amino acids contribution is largely from

mucoproteins which have been reported to be high in glutamic acid, aspartic acid, serine, threonine **(1)**, and proline **(5)**. Hence the difference seen in the flow of different amino acids and with increasing age could be attributed to the decreased rate of mucin secretion with age and/or an increased rate of digestion and absorption of proteins of endogenous origin **(9, 10).** The relative amount of endogenous amino acid flow will be determined by the major source of endogenous secretion into the gut.

At all the ages investigated, CDP method gave higher IEAA and TAA flow estimates when compared to NFD and the regression methods. However, on d 5, estimates from the regression method were higher than that from NFD. The IEAA flow decreased from d 5 to d 15 after which the flow remains relatively constant between d 15 and 21. An increase in the IEAA flow with increasing level of protein in the diet agrees with the findings of **(11)** and **(12)** in growing rats and **(13)** in growing pigs. The effects of negative nitrogen (N) balance on IEAA flow in pigs have been studied **(13,14)**. Findings from these studies showed that negative body N balance does not lead to a lowered endogenous lysine (and amino acids in general) loss when NFD was fed with parenteral infusion of balanced amino acids or saline. Dietary peptides and proteins, and not dietary free amino acids, in the GIT have been reported to be the major sources of increased IEAA flow, this they accomplish by their ability to stimulate the gastro-intestinal tract resulting in an increase in the secretion of protein into the GIT or by inhibiting the digestion and absorption of endogenous protein along the gut.

When the IEAA flow for diets containing four levels of casein was compared (d 5), increasing level of casein in the diets resulted in increasing level of IEAA and TAA flow. This observation supports the fact that increasing level of dietary protein will result in increased IEAA and TAA secretion as reported in pigs **(13)**, and in rats **(11)**. The trend was the same for all levels of casein investigated in this study with the most abundant amino acids at the terminal ileum being glutamic acid >aspartic acid>leucine and the least were tryptophan<methionine. On d 15 the trend for NFD was glutamic acid >aspartic acid>threonine while it was glutamic acid >aspartic acid>serine for 50, 100, and 150 g casein/kg diet. Although the trend was similar to what was seen on d 5, there was a significant reduction in IEAA and TAA flow from d 5 to d 15 (about 50% reduction). As the birds progressed in age, there was a slight shift in the trend of major contributor to IEAA and TAA flow to glutamic acid >aspartic acid>serine for NFD and glutamic acid >serine>aspartic acid for the casein diets with the least contributors to the flow being methionine<histidine. These predominant endogenous amino acids are in agreement with what was reported for pigs and chickens, respectively **(15,16)**. Since these amino acids are the predominant amino acids in mucin proteins **(10)** it could be inferred that mucin contribution to the endogenous amino acids in this study was significant **(17).** The IEAA flow determined with the regression method was higher when compared with NFD method on d 5; however, values on d 15 and 21 for the two methods were similar. The coefficient of variation (CV) between the two methods for lysine, threonine, methionine, glutamic acid, aspartic acid, and TAA was between 12 and 27% on d 5, 1 and 18% on d 15, and 8 and 23% on d 21.

This study confirmed the early results in growing pigs **(18)** that the presence and levels of amino acids or protein in diets may lead to increased IEAA flow. At the ages evaluated in this study, NFD method resulted in lower IEAA and TAA estimation. On d 5 and d 21, cysteine is the only amino acid whose level in the digesta did not change with increasing dietary casein level (50 to 150 g/kg diet).

Out of all the levels of casein evaluated in this study, the NFD method was the most consistent (across laboratories) as there was no significant interaction between location and age for any of the amino acids. Despite the fact that interaction between location and age was significant when 50, 100, or 150 g casein diet was fed, the mean values for IEAA flow for all the amino acids on d 5 and 21 were similar. For example, the endogenous methionine flow on d 21 when four levels of casein were fed were 44 (loc 1) vs. 55 (loc 2) for NFD, 91 (loc 1) vs. 109 (loc 2) for 50g casein diet, 146 (loc 1) vs. 153 (loc 2) for 100 g casein diet, 208 (loc 1) vs. 282 (loc 2) for 150 g casein diet; with a SD of 27.4 (loc 1) and 35.5 (location 2) for methionine.

The results from this study also shows a lack of interaction between location and diet when NFD was fed. In the same vein, there was no location by diet interaction on d 5 when four levels of casein were fed. However, on d 15 and 21 there was significant interaction between location and diet for some of the amino acids although the trends were similar in both locations. The observed interaction could be as a result of difference in the standard deviation which was two times greater in location 1 compared to location 2 for some of the amino acids. This shows that differences between sampling across laboratories may have contributed to the interaction observed.

The conclusion from this study is that it may be important to re-evaluate the basis on which apparent digestibility method is the standard way of evaluating amino acid digestibility of feed ingredients. The high levels of amino acid of endogenous origin, especially on d 5, may be responsible for the effects of age on amino acid digestibility within the first three weeks. Based on the apparent digestibility values, it is believed that nutrient digestibility and absorption at early ages is not as efficient as at later ages, the question is whether or not such a conclusion will still be valid after basal endogenous amino acid contribution to ileal digesta is corrected for. Also, the IEAA and TAA flow obtained from the regression method is similar to values obtained from the NFD (d 15 and 21) method relative to the CDP method.

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Table 1 Dietary composition of experimental diets (on as fed basis)

	Dietary casein, g/kg			
Ingredient g/kg	0	50	100	150
Corn starch	169.0	119.0	69.0	19.0
Dextrose	640.0	640.0	640.0	640.0
Casein	0.0	50.0	100.0	150.0
Solkafloc	50.0	50.0	50.0	50.0
Soyabean oil	50.0	50.0	50.0	50.0
Vitamin-Mineral Premix[1]	5.0	5.0	5.0	5.0
Dicalcium phosphate	19.0	19.0	19.0	19.0
NaHCO3	20.0	20.0	20.0	20.0
KCl	12.0	12.0	12.0	12.0
MgO	2.0	2.0	2.0	2.0
Choline chloride	3.0	3.0	3.0	3.0
Limestone	13.0	13.0	13.0	13.0
NaCl	2.0	2.0	2.0	2.0
Chromic Ox Premix[2]	15.0	15.0	15.0	15.0
Total	1000.0	1000.0	1000.0	1000.0
Calculated nutrients				
ME_n, kcal/kg	*3,618.0*	*3,621.0*	*3,624.0*	*3,627.0*
CP, g/kg	*0*	*43.6*	*87.2*	*130.8*
Ca, g/kg	*9.2*	*9.5*	*9.8*	*10.1*
nPP, g/kg	*4.5*	*5.0*	*5.5*	*6.0*

[1]Provided per kg of diet: Iron, 71.6 mg; Copper, 11.0 mg; Manganese, 178.7 mg; Zinc, 178.7 mg; Iodine, 3.0 mg; Selinium, 0.4 mg. Vitamin A (retinyl acetate), 18,904.3 IU; Vitamin D_3 (cholecalciferol), 9,480.0 IU; Vitamin E (DL-α-tocopheryl acetate), 63.0 IU; Vitamin K activity, 6.4 mg; Thiamine, 3.2 mg; Riboflavin, 9.4 mg; Pantothenic acid, 34.7 mg; Niacin, 126.0 mg; Pyridoxine, 4.7 mg; Folic acid, 1.6 mg; Biotin, 0.5 mg; Vitamin B_{12}, 35.4 mcg; choline, 956.9 mg.

[2]Chromic oxide (Cr_2O_3) premix added as index at a ratio 1:4 of chromic oxide:corn starch.

Table 2 Analyzed dietary composition of nitrogen-free diet (0 g casein /kg diet) and diets containing graded levels of casein (on DM basis)

	Dietary casein, g/kg			
Item	0	50	100	150
Essential amino acids				
Arginine	0.00	1.41	3.15	5.00
Histidine	0.00	1.19	2.61	4.13
Isoleucine	0.00	2.17	4.56	7.28
Leucine	0.00	4.12	8.58	13.69
Lysine	0.20	3.47	7.38	12.06
Methionine	0.00	0.98	2.28	3.69
Phenylalanine	0.00	2.06	4.45	7.61
Threonine	0.00	1.63	3.47	5.54
Tryptophan	<0.40	0.65	1.11	1.74
Valine	0.00	2.82	5.97	9.45
Nonessential amino acids				
Alanine	0.00	1.30	2.71	4.35
Aspartic acid	0.00	3.04	6.40	10.21
Cysteine	0.00	0.22	0.43	0.65
Glutamic acid	0.10	9.55	19.97	31.73
Glycine	0.00	0.76	1.63	2.61
Proline	0.00	0.45	0.96	1.48
Serine	0.00	2.17	4.34	6.84
Tyrosine	0.00	1.41	3.91	6.74
Total amino acid	1.52	44.48	93.46	148.96

Table 3 Ileal endogenous amino acids and total amino acid flow (mg/kg DM intake) in chicks fed nitrogen-free diet at two experimental locations

Item	Day 5	Day 15	Day 21	SD	Location*age
N[1]	8	11	10		
Essential amino acid flow, mg/kg DMI					
Arginine	437^{a}	156^{b}	168^{b}	56.1	0.2070
Histidine	182^{a}	71^{b}	73^{b}	23.2	0.3134
Isoleucine	375^{a}	153^{b}	162^{b}	50.2	0.4495
Leucine	633^{a}	241^{b}	251^{b}	81.4	0.2680
Lysine	485^{a}	178^{b}	181^{b}	64.5	0.2132
Methionine	154^{a}	51^{b}	50^{b}	22.0	0.5323
Phenylalanine	390^{a}	154^{b}	154^{b}	53.9	0.3475
Threonine	539^{a}	274^{b}	274^{b}	55.5	0.1216
Valine	494^{a}	205^{b}	214^{b}	63.4	0.3121
Nonessential amino acid flow, mg/kg DMI					
Alanine	436^{a}	170^{b}	177^{b}	52.6	0.4325
Aspartic acid	799^{a}	337^{b}	340^{b}	95.0	0.2176
Cysteine	227^{a}	115^{b}	136^{b}	26.0	0.2974
Glutamic acid	1,000^{a}	383^{b}	420^{b}	122.6	0.6447
Glycine	413^{a}	195^{b}	205^{b}	47.0	0.1217
Proline	427^{a}	215^{b}	240^{b}	50.8	0.2137
Serine	504^{a}	230^{b}	260^{b}	62.1	0.3658
Tyrosine	281^{a}	116^{b}	124^{b}	41.5	0.3430
Total amino acid	8,692^{a}	3,730^{b}	3,952^{b}	1,012.2	*0.3683*

[a,b] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[1] Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).

Table 4 Ileal endogenous amino acids and total amino acid flow (mg/kg DM intake) in chicks fed diet containing 50 g casein/kg diet at two experimental locations

	Day				
	5	15	21		
Item				SD	Location*age
N[1]	8	8	10		
Essential amino acids flow, mg/kg DMI					
Arginine	678[a]	222[b]	236[b]	56.7	0.0110
Histidine	308[a]	117[b]	124[b]	30.4	0.0004
Isoleucine	744[a]	343[b]	368[b]	72.0	0.0112
Leucine	1,070[a]	363[b]	378[b]	91.9	0.0067
Lysine	873[a]	315[b]	305[b]	94.6	0.0012
Methionine	276[a]	99[b]	100[b]	21.7	0.0258
Phenylalanine	624[a]	207[b]	209[b]	54.5	0.0287
Threonine	833[a]	417[b]	387[b]	72.0	0.0017
Valine	874[a]	370[b]	392[b]	80.6	0.0024
Nonessential amino acids flow, mg/kg DMI					
Alanine	677[a]	271[b]	285[b]	52.4	0.0012
Aspartic acid	1,326[a]	568[b]	551[b]	117.8	0.0042
Cysteine	289[a]	154[c]	184[b]	30.4	0.0465
Glutamic acid	2,329[a]	1,015[b]	1,075[b]	232.6	0.0128
Glycine	598[a]	275[b]	297[b]	50.0	0.0003
Proline	843[a]	372[b]	383[b]	92.3	0.0130
Serine	922[a]	518[b]	559[b]	85.2	0.0030
Tyrosine	505[a]	173[b]	173[b]	51.0	0.0751
Total amino acid	14,848[a]	6,387[b]	6,550[b]	1,311.1	*0.0042*

[a,b,c] Means within the same row with different superscripts are significantly different, $P < 0.05$

[1] Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).

Table 5 Ileal endogenous amino acids and total amino acid flow (mg/kg DM intake) in chicks fed diet containing 100 g casein/kg diet at two experimental locations

Item	Day 5	Day 15	Day 21	SD	Location*age
N[1]	9	8	12		
Essential amino acids flow, mg/kg DMI					
Arginine	727^{a}	262^{b}	286^{b}	80.3	0.1311
Histidine	369^{a}	161^{b}	172^{b}	38.2	0.0032
Isoleucine	893^{a}	593^{b}	557^{b}	87.8	0.0057
Leucine	1,218^{a}	475^{b}	505^{b}	115.5	0.1327
Lysine	1,050^{a}	432^{b}	400^{b}	116.6	0.0101
Methionine	327^{a}	138^{b}	149^{b}	31.3	0.0365
Phenylalanine	690^{a}	235^{b}	259^{b}	76.2	0.1166
Threonine	945^{a}	535^{b}	470^{b}	86.1	0.0797
Valine	1,027^{a}	588^{b}	540^{b}	104.0	0.0469
Nonessential amino acids flow, mg/kg DMI					
Alanine	759^{a}	371^{b}	367^{b}	73.4	0.0732
Aspartic acid	1,510^{a}	771^{b}	736^{b}	137.1	0.0426
Cysteine	292^{a}	175^{b}	213^{b}	35.6	0.3847
Glutamic acid	2,937^{a}	1,868^{c}	1,681^{b}	291.3	0.0015
Glycine	650^{a}	330^{b}	357^{b}	62.4	0.1581
Proline	1,029^{a}	511^{b}	520^{b}	94.3	0.0232
Serine	1,105^{a}	883^{b}	835^{b}	122.7	0.0197
Tyrosine	568^{a}	207^{b}	227^{b}	58.7	0.2732
Total amino acid	17,129^{a}	9,118^{b}	8,832^{b}	1,515.6	0.0270

^{a,b} Means within the same row with different superscripts are significantly different, $P < 0.05$.
[1] Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).

Table 6 Ileal endogenous amino acids and total amino acid flow (mg/kg DM intake) in chicks fed diet containing 150 g casein/kg diet at two experimental locations

Item	Day 5	Day 15	Day 21	SD	Location*age
N[1]	9	7	10		
Essential amino acids flow, mg/kg DMI					
Arginine	833^{a}	291^{c}	415^{b}	122.3	0.0037
Histidine	458^{a}	196^{c}	259^{b}	60.7	0.0004
Isoleucine	1,128^{a}	728^{c}	871^{b}	136.5	0.0035
Leucine	1,455^{a}	543^{c}	762^{b}	186.8	0.0051
Lysine	1,302^{a}	557^{c}	706^{b}	185.1	0.0035
Methionine	392^{a}	169^{b}	245^{b}	50.1	0.0418
Phenylalanine	808^{a}	258^{c}	70^{b}	104.5	0.0076
Threonine	1,148^{a}	619^{b}	771^{b}	161.3	0.0017
Valine	1,280^{a}	698^{b}	841^{b}	160.7	0.0041
Nonessential amino acids flow, mg/kg DMI					
Alanine	909^{a}	437^{c}	562^{b}	117.8	0.0023
Aspartic acid	1,856^{a}	938^{b}	1,166^{b}	234.8	0.0034
Cysteine	325^{a}	194^{c}	266^{b}	47.9	0.0048
Glutamic acid	3,828^{a}	2,309^{c}	2,870^{b}	462.0	0.0067
Glycine	780^{a}	373^{c}	505^{b}	113.0	0.0018
Proline	1,371^{a}	604^{c}	830^{b}	170.0	0.0043
Serine	1,390^{a}	1,086^{b}	1,310^{a}	193.0	0.0037
Tyrosine	668^{a}	236^{c}	332^{b}	85.1	0.0122
Total amino acid	20,950^{a}	10,989^{c}	13,923^{b}	2,581.3	*0.0037*

[a,b,c] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[1] Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).

Table 7 Ileal endogenous amino acids flow (mg/kg DM intake) in chicks fed four levels of casein (day 5, at two experimental locations locations)

Item	Dietary casein, g/kg 0	50	100	150	SD[2]	Linear effect[1]	Probability Location*diet
N[2]	8	8	9	9			
Essential amino acid flow, mg/kg DMI							
Arginine	437	678	727	833	98.4	< 0.0001	0.4423
Histidine	182	308	369	458	54.3	< 0.0001	0.1365
Isoleucine	375	744	893	1,128	98.4	< 0.0001	0.6855
Leucine	633	1,070	1,218	1,455	154.1	< 0.0001	0.6929
Lysine	485	873	1,050	1,302	158.7	< 0.0001	0.2656
Methionine	154	276	327	392	38.7	< 0.0001	0.4529
Phenylalanine	390	624	690	808	97.5	< 0.0001	0.4990
Threonine	539	833	945	1,148	99.1	< 0.0001	0.4937
Tryptophan	64	85	94	115	12.2	< 0.0011	0.8717
Valine	494	874	1,027	1,280	129.3	< 0.0001	0.4621
Nonessential amino acid flow, mg/kg DMI							
Alanine	436	677	759	909	90.5	< 0.0001	0.5737
Aspartic acid	799	1,326	1,510	1,856	177.1	< 0.0001	0.5786
Cysteine	227	289	292	325	38.4	0.0016	0.1280
Glutamic acid	1,000	2,329	2,937	3,828	310.8	< 0.0001	0.5539
Glycine	413	598	650	780	77.1	< 0.0001	0.4704
Proline	427	843	1,029	1,371	138.1	< 0.0001	0.3154
Serine	504	922	1,105	1,390	102.4	< 0.0001	0.4328
Tyrosine	281	505	568	668	75.9	< 0.0001	0.4646
Total amino acid	8,692	14,848	17,129	20,950	1,921.9	< 0.0001	0.5495

[1] Linear effects, $P < 0.05$.
[2] Number of replicates. Each cage represents an experimental unit with 30 birds per cage.

Table 8 Ileal endogenous amino acids flow (mg/kg DM intake) in chicks fed four levels of casein diets (day 15 at two experimental locations)

Item	Dietary casein, g/kg 0	50	100	150	SD	Linear effect[1]	Probability Location*diet
N[2]	11	8	8	7			
Essential amino acid flow, mg/kg DMI							
Arginine	156	222	262	291	69.1	*.0002*	0.1840
Histidine	71	117	161	196	30.2	<*.0001*	0.0114
Isoleucine	153	343	593	728	76.6	<*.0001*	0.0002
Leucine	241	363	475	543	96.3	<*.0001*	0.0969
Lysine	178	315	432	557	98.7	<*.0001*	0.0012
Methionine	51	99	138	169	20.4	<*.0001*	0.0096
Phenylalanine	154	207	235	258	63.6	*.00016*	0.3933
Threonine	274	417	535	619	93.8	<*.0001*	0.0339
Valine	205	370	588	698	96.9	<*.0001*	0.0777
Nonessential amino acid flow, mg/kg DMI							
Alanine	170	271	371	437	63.1	<*.0001*	0.0211
Aspartic acid	337	568	778	938	136.1	<*.0001*	0.0109
Cysteine	115	154	175	194	38.8	*.0001*	0.1613
Glutamic acid	383	1,015	1,868	2,369	248.9	<*.0001*	<0.0001
Glycine	195	275	330	373	61.9	<*.0001*	0.0976
Proline	215	372	511	604	86.9	<*.0001*	0.0282
Serine	230	518	883	1,086	116.7	<*.0001*	<0.0001
Tyrosine	116	173	208	236	56.7	<*.0001*	0.3447
Total amino acid	3,730	6,387	9,118	10,989	1,454.9	<*.0001*	0.0052

[1]Linear effects, $P < 0.05$.
[2]Number of replicates. Each cage represents an experimental unit with 30 birds per cage.

Table 9 Ileal endogenous amino acids flow (mg/kg DM intake) in chicks fed four levels of casein diets (day 21, at two experimental locations)

Item	Dietary casein, g/kg 0	50	100	150	SD	Linear effect[1]	Probability Location*diet
N[2]	10	10	12	10			
Essential amino acid flow, mg/kg DMI							
Arginine	168	236	286	415	78.4	<*.0001*	0.0757
Histidine	73	124	172	259	33.2	<*.0001*	0.0265
Isoleucine	162	368	557	871	95.6	<*.0001*	0.0133
Leucine	251	378	505	762	117.6	<*.0001*	0.0598
Lysine	181	305	400	706	103.5	<*.0001*	0.1404
Methionine	50	100	149	245	35.9	<*.0001*	0.0769
Phenylalanine	154	209	259	370	60.6	*.00016*	0.1477
Threonine	274	387	470	771	106.6	<*.0001*	0.0244
Valine	214	392	540	841	95.4	<*.0001*	0.0474
Nonessential amino acid flow mg/kg DMI							
Alanine	177	285	367	562	77.7	<*.0001*	0.0328
Aspartic acid	340	551	736	1,166	146.2	<*.0001*	0.0469
Cysteine	136	184	213	266	30.4	*.0001*	0.0741
Glutamic acid	420	1,075	1,681	2,870	324.2	<*.0001*	0.0096
Glycine	205	297	357	505	75.4	<*.0001*	0.0465
Proline	240	383	520	830	97.3	<*.0001*	0.0190
Serine	260	559	835	1,301	144.4	<*.0001*	0.0088
Tyrosine	124	173	227	332	49.0	<*.0001*	0.2406
Total amino acid	3,952	6,550	8,832	13,923	1,661.0	<*.0001*	0.0273

[1]Linear effects, $P < 0.05$.

[2]Number of replicates. Each cage represents an experimental unit with 8 birds per cage.

Table 10. Ileal endogenous amino acid and total amino acid flow in broiler chicks fed graded levels of casein from regression method when the regression line is extrapolated to zero percent casein in diet (data from only one location)

Item	Amino acid flow, mg/kg[1]								
	Day 5			Day 15			Day 21		
		SD	P-value[2]		SD	P-value		SD	P-value
Essential amino acid flow, mg/kg DMI									
Arginine	589	99.8	0.00074	185	81.9	0.27983	132	88.5	0.28093
Histidine	228	57.7	0.00730	74	37.1	0.46100	48	37.9	0.17045
Isoleucine	535	103.6	0.00017	137	86.9	0.38446	93	111.1	0.11009
Leucine	860	158.7	0.00054	265	116.8	0.38466	162	134.2	0.14855
Lysine	643	169.6	0.00225	176	125.2	0.50000	65	126.2	0.04198
Methionine	216	38.3	0.00104	60	24.1	0.27983	19	41.7	0.04755
Phenylalanine	522	99.9	0.00134	178	72.9	0.31306	117	68.2	0.24994
Threonine	657	105.0	0.00161	303	115.7	0.38489	154	129.9	0.11138
Tryptophan	NA	NA	NA	NA	NA	NA	NA	NA	NA
Valine	651	136.1	0.00161	197	120.9	0.3855	139	112.5	0.14855
Nonessential amino acid flow, mg/kg DMI									
Alanine	547	91.4	0.00382	180	77.4	0.42241	125	90.5	0.19464
Aspartic acid	1,029	186.3	0.00107	362	168.4	0.42241	197	172.7	0.11138
Cysteine	265	39.7	0.00910	131	44.4	0.34841	139	33.1	0.46127
Glutamic acid	1,524	339	0.00001	285	271.3	0.2181	67	391.3	0.01543
Glycine	4912	81.1	0.00001	219	74.2	0.34841	176	85.5	0.34878
Proline	548	154.5	0.00230	241	103.6	0.34809	127	116.7	0.09588
Serine	686	107.7	0.00012	210	133.9	0.38446	145	168.2	0.11009
Tyrosine	416	79.6	0.00004	136	65.2	0.31272	83	54.4	0.17045
Total amino acid	11,492	2,022.2	0.00039	3,851	1,783.4	0.46100	2,329	1,965.9	0.11138

[1] Data from the two locations for d 5 and 21 were used in the regression equation calculation, however, only the data from location1 were used for d 15 calculation.
[2] Probability values for the comparison of ileal endogenous amino acid flow for the NFD and the regression methods

(Paper 3: To be submitted to Poultry Science Journal)

Running Title: ENDOGENOUS AMINO ACID FLOW IN TURKEY POULTS

Section: Metabolism and nutrition

Effect of Age, Method, and Location on Ileal Endogenous Amino Acid and Total Amino Acid Flows in Turkey Poults [1]

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Abbreviation Key: CDP = Completely digestible protein, DMI = Dry matter intake, IEAA = Ileal endogenous amino acid, NFD = Nitrogen-free diet, TAA = Total amino acid

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Section: Metabolism and nutrition

Effect of age, method, and location on ileal endogenous amino acid and total amino acid flows in turkey poults

ABSTRACT Ileal endogenous amino acid (IEAA) and total amino acid (TAA) flows in turkey poults were determined at two experimental locations on d 5, d 15, and d 21 using three methods including a nitrogen-free diet (NFD), a completely digestible protein (casein; CDP), and the regression method-obtained by regressing dietary casein levels against IEAA or TAA flow. The diets were semi-purified and contained 0, 50, 100, or 150 g casein/kg diet as the sole source of dietary protein. Each diet was fed for 5-d to six replicate cages of 30 (d 5), 10 (d 15), or 8 (d 21) birds per cage prior to sampling. There was no interaction between locations and age or locations and diet so the data from both locations were pooled. Ileal EAA flow on d 5 (NFD method) was higher than on d 15 and d 21 *($P < 0.05$)* and there were no differences between d 15 and d 21. Flows estimated from the NFD and the regression methods were different on d 5 *($P < 0.05$)* but there were no differences in IEAA flow for most of the amino acids on d 15 and d 21.Total IEAA flow in mg/kg DMI when the NFD was fed was 19,227 (d 5), 6,429 (d 15) and 6,843 (d 21). Increasing the level of casein resulted in a linear *($P < 0.05$)* increase in IEAA and TAA flow *($P < 0.05$)*. The amino acids with the lowest flow were tryptophan and methionine while glutamic acid had the greatest flow. The results obtained from this study indicate that results across different locations were repeatable. The results also suggest that as chicks age, there is a decrease in IEAA and TAA flow and this stabilizes between d 15 and d 21. This observation suggests that apparent digestibility coefficients for poults on d 5 and d 15 or d 21 are significantly influenced by the level endogenous amino acids, especially on d 5.

(*Key words*: Casein, endogenous amino acid, nitrogen-free diet, turkey poult)

INTRODUCTION

The need to optimize amino acid utilization in poultry by formulating diets on a digestible amino acid basis is becoming much more important with the increased focus on decreasing nutrient excretion and this has been discussed in several recent publications (De Lange et al, 2003; Lemme et al., 2004). The advantages include more closely meeting the bird's actual requirements while taking into consideration the digestibility of amino acids from various feed ingredients. This can only be achieved, however, if we are able to discriminate between amino acids of dietary and endogenous origin.

A number of studies have been conducted in chicks and adult chickens of different ages and strains using a variety of methods to estimate amino acids of endogenous origin (Siriwan, et al., 1993; Ravindran and Hendriks, 2004; Ravindran et al., 2004). From these studies, the different methods of estimating IEAA flow resulted in different estimates of endogenous amino acid concentrations. Endogenous amino acid flows from fasted cockerels have been reported to be lower than the flow from birds fed a NFD (Muztar and Slinger, 1980). Likewise, the presence in the gut of increasing levels of dietary amino acids of dietary origin have been reported to have resulted in increasing endogenous amino acid secretion in broilers and cockerels (Siriwan et al., 1993). The origin of endogenous amino acids varies and may include amino acids from the digestive enzymes of salivary, gastric, and billiary origin, mucoproteins, and sloughed cells (Ravindran and Hendriks, 2004).

Given that an apparent digestibility coefficient includes both the basal as well as the diet-induced flow of an endogenous amino acid, a factor is needed to delineate the contribution of amino acids of endogenous origin; hence the determination of IEAA becomes important. There is a lack of data on IEAA and TAA flows in turkey poults at very young ages and likewise, a need to compare IEAA and TAA flows in poults under similar environmental and procedural conditions, but at different experimental locations, to asses the repeatability of the data. The objective of the current study, therefore, was to examine the effects of age, location, and method on basal IEAA flow in turkey poults. These basal values can be subsequently used to determine standardized ileal amino acid digestibility coefficients for different feed ingredients.

MATERIAL AND METHODS

Diet formulation

Four-semi purified diets were formulated to contain graded concentration of protein (amino acids) supplied directly by casein (0, 50, 100, or 150 g/kg diet). The dietary composition of the experimental diets and the analyzed values of the different amino acids are reported in Tables 1 and 2. For the IEAA and TAA flow calculations, chromic oxide was added to the treatment diets at as an indigestible marker at 3 g/kg diet.

Birds and housing

One thousand one hundred and fifty two day-old male turkey poults (Nicholas) were reared at each of the two locations. Poults were obtained from commercial hatcheries and 720 poults were weighed individually and randomly allocated to diets on d 0. Each diet was fed for 5 consecutive days before ileal digests was collected on d 5, d 15, and d 21. Poults in six replicate cages containing 30 chicks per cage were euthanized and the ileal contents removed on d 5. The remaining chicks were fed a conventional corn-soybean meal-based starter diet appropriate for turkey poults that meets the NRC (1994) requirements. At d 10, 240 of the remaining poults were randomly assigned to 6 replicate cages per diet. These poults were euthanized and the ileal contents collected on day 15. On day 16, 192 poults were placed on the experimental diets and ileal contents were collected on day 21. Across all ages, euthanasia was by CO_2 asphyxiation.

Birds were raised in battery cages (Alternative Design Manufacturing and Supply, Inc. Siloam Springs, AR) maintained in an environmentally controlled room with 24-h of light throughout the duration of the study. The room temperature was 35 ^{o}C during the first week and dropped by 5 degrees during each of the subsequent weeks. Birds had free access to feed and water. All animal care procedures were approved by the Purdue University and the Ohio State University Animal Care and Use Committee.

Sampling and ileal digesta processing

On d 5, d 15, and d 21, the contents from the ileal region between Meckel's diverticulum to about 5 mm proximal to the ileo-cecal junction region was flushed with distilled water. For birds sampled on d 5, a 50 mL syringe was used while a wash bottle was used for flushing on d 15 and d 21. The ileal digesta samples from all the poults within a cage were pooled, frozen, and stored at -20 ^{o}C, until they were processed. All frozen samples were freeze-dried and ground using mortar and pestle.

Chemical analysis

The dry matter content was determined on the ground diets and ileal digesta by drying the samples at 100 ^{o}C for 24 h. Amino acids and chromium analyses were conducted at the University of Missouri Experiment Station and Chemical Laboratory. For amino acid analyses, samples were hydrolyzed in a 6 N HCl for 24 h at 110^{o}C under N atmosphere. For the sulfur containing amino acids, methionine and cysteine, performic acid oxidation was carried out before acid hydrolysis. For tryptophan analysis, samples were hydrolyzed using barium hydroxide. The amino acids in the hydrolysate were determined by HPLC after post-column derivatization (AOAC, 2000; 982.30 E [a, b, c]. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Chromium was determined by the inductively coupled plasma atomic emission spectroscopy method (AOAC, 2000; 990.08) following nitric/perchloric acid wet ash digestion.

Calculations

Ileal endogenous amino acid flow and TAA flow were calculated as mg of amino acid or TAA flow per kg of feed intake on DM basis using the formula proposed by Moughan et al. (1992):

Endogenous amino acid or TAA flow (mg/kg DM intake) = [amino acid or TAA in ileal digesta] x (diet chromium, mg/kg/ileal chromium, mg/kg

Statistical analysis

The data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC, 2000). Orthogonal polynomial contrasts were used to compare the treatment means (the effects of dietary casein concentration). Where necessary, treatment means with significant F ratios were separated using Tukey adjustment. Ileal EAA flow was determined by regression method by regressing dietary casein concentration against IEAA or TAA flow.). A comparison of the IEAA and TAA flows between the NFD and regression method was made by calculating the standard errors of difference of means as outlined in Samuels and Witmer (1999). The probabilities of the t-values were determined using the t-test of SAS.

RESULTS

The analyzed dietary amino acids and TAA are reported in Table 2. The level of the dietary TAA increased from 1.2 to 138.5 g/kg diet between 0 (NFD) and 150 g casein/kg diet, respectively (Table 2). There was no significant interaction between experimental location and age and location and diet hence data from both locations were pooled before statistical analyses.

The mean values for IEAA and TAA flow when the NFD was fed as well as estimates from the regression method are presented in Table 3. The IEAA and TAA flow on d 5 was higher *(P < 0.05)* than on d 15 and d 21. The IEAA and TAA flow for most of the amino acids on d 15 and d 21 was less than 40% of the flow on d 5. Methionine and histidine were the amino acids with the lowest values while the flow of glutamic and aspartic acids were the highest. The extrapolated IEAA and TAA flows to zero percent casein in the diet on d 15 and d21 were about 50% of that on d 5 (Table 3). However, IEAA and TAA flow on d 15 and d 21 were similar. The ileal endogenous flows for all the amino acids and TAA on d 5 was different with the NFD method resulting in higher IEAA flow relative to values from the regression method *(P < 0.05)*. On d 15, however, only three amino acids (methionine, threonine, and cysteine) were different *(P < 0.05)*. Theonine, valine, cysteine, glycine, and proline were the only amino acids in addition to TAA whose flows were higher with the NFD method relative to the regression method on d 21 *(P < 0.05)*.

On d 5, d 15, and d 21 IEAA and TAA flow in broiler chicks fed four levels of casein is reported on Tables 4, 5 and 6, respectively. There was a linear effect of casein on IEAA and TAA flow with an increase in flow with increase in dietary casein level *(P < 0.05)*. The interaction between location and diet was not significant on d 5 and d 21. The interaction between location and dietary treatments was not tested on d 15 because data from one of the two locations were discarded from the entire analysis because the collection method stated in the protocol (flushing with distilled water) was not adhered to. On d 5, there was a linear *(P < 0.05)* increase of IEAA flow (Table 4). However, there was a decrease in IEAA and TAA flow between the 0 (NFD) to 50 g casein diets (Table 4). On d 15, IEAA and TAA flow increased linearly with increasing level of dietary amino acids in mg/kg DMI from 6,429 (NFD) to 10,518 (150 g casein/kg diet (Table 5). On d 21, TAA flow increased from 6,842 to 12,078 mg/kg DMI between the 0 (NFD) and 150 g casein/kg diets, respectively.

DISCUSSION

The primary objective of this study was to determine the effects of age, location, and method on IEAA flow in turkey poults. In an effort to be able to formulate diets based on digestible amino acids as advocated by Rostango et al. (1995), De Lange, et al. (2003), and

Lemme et al. (2004), it is imperative that the contribution of amino acids of endogenous origin be determined. For this study, IEAA flow was estimated using three methods in the same experimental setting but in two locations so as to be able to compare the different methods as they relate to each other as well as to explore how consistent these methods are when the study is conducted in two different locations.

The influence of age on IEAA flow is obvious from the results of these studies. Irrespective of the method used, the flow of amino acids of endogenous origin was higher on d 5 than on d 15 or d 21. This was consistent across the three methods used. The, values on d 15 and d 21, however, were not significantly different. The very high output of IEAA on d 5 could be attributed to intestinal secretions of digestive enzymes, sloughed epithelial cells lining the gastro-intestinal tract (Moughan et al., 1992) as well as a rapid rate of intestinal cell proliferation and turnover within the first week of age (Uni, 1999). The fact that the IEAA flow was higher on d 5 or slightly lower on d 15 and d 21 when the NFD method was compared with the 50 g casein/kg diet could be an indication that the presence of protein in the diet increased IEAA flow on d 15 and d 21 while decreasing flow on d 5. It is also evident from the results of this study that increasing level of dietary casein increased the level of IEAA output. This observation is supported by the findings of Brannon (1990) and Siriwan et al. (1993) who suggested that increasing levels of dietary amino acids could increase the endogenous secretion of digestive enzymes as well as increasing sloughing of cells lining the intestinal wall. There is also the likelihood that at a dietary high casein concentration, the amino acids of dietary origin may not be 100% digestible hence contributing to the dose-response relationship observed.

The interaction between location and age was also determined for each of the dietary treatments. It can be inferred from the results of this study that the lack of interaction between location and age, except for threonine (50 g casein/kg diet), supports the conclusion that the methods of determining IEAA flow used in this study are repeatable across experimental locations. The lack of interaction between location and age is strongest for the NFD method which indirectly suggests that it could be the method (NFD) that is most likely to be repeatable and consistent across laboratories.

When amino acids of endogenous origin were determined by extrapolating a regression line to zero percent protein intake, values obtained for d 5 were 30% less than the values obtained using the NFD method. On d 15 and d 21, the regression method IEAA flows were

2.9% and 17.0% less than that of the NFD method. These results show that in 15-d or 21-d old turkey poults, estimates of IEAA flow using either the regression or NFD method will be similar. This observation is different from what Siriwan et al. (1993) reported in 5-wk-old broiler. They observed a significantly higher IEAA flow when the regression method was used relative to the NFD method. This again, is an indication that the NFD method, despite its criticism of not being physiological, closely mirrors that of the regression method and appears to be more reliable even when such data are generated from other laboratories.

Increasing concentration of casein in the diets from 0 to 150 g/kg diet resulted in a linear increase in IEAA flow. When the IEAA from the 21-d old poults used in this study was compared to the IEAA flow in 5-wk-old broilers (Ravindran et al., 2004), a similar trend was observed, especially when using the NFD method. However, when the 150 g casein/kg diet in the current study was compared to the diets containing 190 g enzyme hydrolyzed casein and 194 g guanidinated casein (Ravindran et al., 2004) the IEAA flow in the chicks (Ravindran et al, 2004) was higher than what was observed for poults in this study. A number of things may have contributed to this including age differences, species differences and differences in the levels of dietary crude protein. Most of the individual IEAA flows values determined using the NFD methods were similar in both studies. The level of ileal TAA flow in 21-d-old broiler chicks fed the 150 g casein/kg diet in this study (12,078 mg/kg DMI) is similar to what was reported by Ravindran and Hendriks (2004) for 6-wk-old broiler chicks using the peptide alimentation method (12,305 mg/kg DMI). Likewise, there was no interaction between location and dietary treatments which underscores the fact that in birds of similar age, fed the same diet, and using similar analytical procedures, the effect of experimental location is not significant.

Glutamic acid, aspartic acid, leucine, threonine, valine, proline, and serine were the amino acids with the highest endogenous flow, independent of the method used. These amino acids have been reported to be high in mucin and since little digestion of mucin takes place before the distal end of the gastrointestinal tract (Lien et al., 1997), it would be expected that their concentrations in the digesta would be fairly high relative to the other amino acids. An increase in these amino acids, especially, threonine, with an increasing concentration of dietary casein may be an indication of an increase in the level of mucin production with increasing dietary amino acids. This could be due to the need to protect the intestinal lining from the increasing levels of digestive enzymes being secreted in response to increased level of dietary proteins in the gut.

The results from the comparison of the two methods (NFD and the regression) suggest that at the younger age (d 5) the two methods will give different results with the NFD method resulting in a higher flow estimate. At older ages, however, the two methods resulted in similar endogenous amino acid flow with the exception of a few amino acids. With the exception of these amino acids (methionine - d 15; cysteine and glycine - d 21) the NFD method resulted in higher flow for the other amino acids that showed significant difference. This observation is difficult to explain as it is expected that the regression method will result in a higher flow due to the presence of casein in the diets. However, flows from 50 and 150 g casein/kg diet will greatly influence the extrapolated flow from the regression method.

In summary, age can have a significant effect on IEAA flow, with d 5 flows being approximately half of that observed on d 15 or d 21. It is interesting to note, however, that in this study, IEAA flow on d 21 compared favorably with previously published IEAA flow in 5-and 6-wk old broilers. This may be an indication that IEAA flow on DMI between wk 2 and wk 6 may not be very different. This also suggest that what happens at a very early age (<10 days) may be important in controlling the levels of nutrients that are excreted into the environment. Ileal EAA flow is also method dependent with an increase in IEAA flow concomitant with increasing concentration of casein in the diets; with the exception of 0% casein (NFD) which resulted in a higher flow on d 5 compared to the 50 g casein/kg diet. A NFD and the regression methods resulted in flow estimates of different magnitude on d 5 with higher flows from the NFD method but there were no differences on d 15 and d 21 for most amino acids. Only cysteine and threonine showed significant differences in flow at the three ages studied in this experiment. The results from this study also showed that an effect due to location was not a factor as indicated by the high *P*-values.

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Table 1. Dietary composition of experimental diets (on as fed basis)

	Dietary casein, g/kg			
Ingredient g/kg	0	50	100	150
Corn starch	169.0	119.0	69.0	19.0
Dextrose	640.0	640.0	640.0	640.0
Casein	0.0	50.0	100.0	150.0
Solkafloc	50.0	50.0	50.0	50.0
Soyabean oil	50.0	50.0	50.0	50.0
Vitamin-Mineral Premix[1]	5.0	5.0	5.0	5.0
Dicalcium phosphate	19.0	19.0	19.0	19.0
NaHCO3	20.0	20.0	20.0	20.0
KCl	12.0	12.0	12.0	12.0
MgO	2.0	2.0	2.0	2.0
Choline chloride	3.0	3.0	3.0	3.0
Limestone	13.0	13.0	13.0	13.0
NaCl	2.0	2.0	2.0	2.0
Chromic Ox Premix[2]	15.0	15.0	15.0	15.0
Total	1000.0	1000.0	1000.0	1000.0
Calculated nutrients				
ME_n, kcal/kg	*3,618.0*	*3,621.0*	*3,624.0*	*3,627.0*
CP,g/kg	*0.0*	*43.6*	*87.2*	*130.8*
Ca, g/kg	*9.2*	*9.5*	*9.8*	*10.1*
nPP, g/kg	*4.5*	*5.0*	*5.5*	*6.0*

[1]Provided per kg of diet: Iron, 71.6 mg; Copper, 11.0 mg; Manganese, 178.7 mg; Zinc, 178.7 mg; Iodine, 3.0 mg; Selinium, 0.4 mg. Vitamin A (retinyl acetate), 18,904.3 IU; Vitamin D_3 (cholecalciferol), 9,480.0 IU; Vitamin E (DL-α-tocopheryl acetate), 63.0 IU; Vitamin K activity, 6.4 mg; Thiamine, 3.2 mg; Riboflavin, 9.4 mg; Pantothenic acid, 34.7 mg; Niacin, 126.0 mg; Pyridoxine, 4.7 mg; Folic acid, 1.6 mg; Biotin, 0.5 mg; Vitamin B_{12}, 35.4 mcg; choline, 956.9 mg.

[2]Chromic oxide (Cr_2O_3) premix added as index at a ratio 1:4 of chromic oxide:corn starch.

Table 2. Analyzed dietary composition of nitrogen-free diet (0 g casein /kg diet) and diets containing graded levels of casein (on DM basis)

	Dietary casein, g/kg			
	0	50	100	150
Essential amino acid				
Arginine	0.00	1.53	3.38	4.58
Histidine	0.00	1.31	2.83	3.88
Isoleucine	0.00	2.29	5.01	6.76
Leucine	0.11	4.25	9.48	12.87
Lysine	0.11	3.49	7.74	10.47
Methionine	0.00	1.20	2.62	3.38
Phenylalanine	0.00	2.29	5.01	6.87
Threonine	0.00	1.74	3.92	5.24
Tryptophan	<0.40	0.65	1.20	1.75
Valine	0.11	2.94	6.43	8.62
Nonessential amino acid				
Alanine	0.00	1.42	2.94	4.04
Aspartic acid	0.11	3.16	6.87	9.38
Cysteine	0.00	0.22	0.55	0.55
Glutamic acid	0.11	9.91	21.91	29.56
Glycine	0.00	0.89	1.85	2.51
Proline	0.11	4.79	10.57	14.51
Serine	0.00	2.29	5.23	7.09
Taurine	0.54	0.54	0.44	0.44
Tyrosine	0.00	1.53	4.25	6.11
Total amino acid	1.20	46.41	102.26	138.53

Table 3. Ileal endogenous amino acid and total amino acid flows from turkey poults fed nitrogen-free diet and flows from the regression method when the regression line was extrapolated to zero percent casein in diet

	Amino acid flow, mg/kg DMI[1]									
	Day 5			Day 15			Day 21			
	NFD[2]	Reg[3]	SD^{c}	NFD	Reg	SD	NFD	Reg	SD	SD[4]
N[5]	11			6			12			
Essential amino acid flow, mg/kg DMI										
Arginine	1,007^{a}	648	364.7	274^{b}	286	61.2	272^{b}	248	135.6	250.7
Histidine	503^{a}	337	159.1	138^{b}	141	35.7	158^{b}	138	72.9	119.3
Isoleucine	873^{a}	569	327.2	264^{b}	281	117.7	242^{b}	231	131.3	197.3
Leucine	1,564^{a}	1,029	564.0	414^{b}	432	114.1	408^{b}	358	202.0	375.4
Lysine	1,123^{a}	686	449.4	276^{b}	244	124.8	273^{b}	239	160.7	303.6
Methionine	411^{a}	258	161.4	86^{b}	101	27.8^{d}	90^{b}	92	51.5	104.5
Phenylalanine	929^{a}	613	320.1	258^{b}	263	58.7	263^{b}	225	119.4	213.5
Threonine	1,141^{a}	786	355.8	427^{b}	379	82.2^{d}	454^{b}	376	166.2^{c}	229.0
Tryptophan	155	82	73.0	NA	NA	NA	71	NA	NA	54.9
Valine	1,223^{a}	840	438.2	409^{b}	399	115.2	400^{b}	331	160.6^{c}	269.8
Nonessential amino acid flow, mg/kg DMI										
Alanine	1,087^{a}	753	360.5	307^{b}	307	88.2	308^{b}	273	151.6	255.5
Aspartic acid	1,852^{a}	1,227	634.6	560^{b}	561	145.0	568^{b}	503	266.4	420.0
Cysteine	522^{a}	382	132.4	211^{b}	231	32.9^{d}	212^{b}	186	51.7^{c}	88.0
Glutamic acid	2,381^{a}	1,544	913.1	707^{b}	753	330.7	687^{b}	283	128.2^{c}	574.7
Glycine	957^{a}	653	291.8	336^{b}	339	74.6	324^{b}	672	397.9^{c}	207.9
Proline	980^{a}	674	320.2	426^{b}	396	72.1	381^{b}	309	134.9^{c}	200.4
Serine	1,104^{a}	722	341.3	385^{b}	378	138.5	401^{b}	384	164.6	231.3
Tyrosine	618^{a}	407	219.5	188^{b}	192	43.3	193^{b}	166	85.5	134.8
Total amino acid	19,227^{a}	12,766	6,421.5	6,429^{b}	6,243	1,648.2	6,843^{b}	5,657	2,700.6^{d}	4,283.5

^{a,b} Means within the same row with different superscripts are significantly different, $P < 0.05$.
^{c} Comparison between nitrogen-free diet and the regression method within an age were significantly different (d 5, $P < 0.01$; d 21, $P < 0.05$).
[1] Data from the two locations for d 5, d 15, and 21 were used in the regression equation calculation. Each replicate is one cage and each cage represents an experimental unit with 30 birds per cage (d 5), 10 birds per cage (d 15), and 8 birds per cage (d 21).
[2] Nitrogen-free diet.
[3] Regression method.
[4] Overall standard deviation for age comparison for the nitrogen-free diet method.
[5]Number of replicates.

Table 4. Ileal endogenous amino acids and total amino acid flow (mg/kg DM intake) in poults fed four levels of casein (d 5, mean for the two experimental locations)

Item	Dietary casein, g/kg 0	50	100	150	SD	Linear effect[1]	Probability Location*diet[2]
N[3]	11	11	10	8			
Essential amino acid flow, mg/kg DMI							
Arginine	1,007	784	1,103	1,274	379.4	0.05	NS[3]
Histidine	503	395	553	619	169.5	0.04	NS
Isoleucine	873	734	1,056	1,250	328.3	<0.01	NS
Leucine	1,569	1,251	1,760	2,038	582.4	0.03	NS
Lysine	1,123	888	1,341	1,568	463.4	0.01	NS
Methionine	411	323	467	542	164.2	0.03	NS
Phenylalanine	929	734	1,029	1,173	330.0	0.04	NS
Threonine	1,141	941	1,289	1,446	359.6	0.02	NS
Tryptophan	155	124	169	210	73.9	0.06	NS
Valine	1,223	1,027	1,403	1,622	441.9	0.02	NS
Nonessential amino acid flow, mg/kg DMI							
Alanine	1,087	882	1,222	1,371	378 7	0.04	NS
Aspartic acid	1,852	1,500	2,087	2,406	651.3	0.02	NS
Cysteine	522	414	532	561	134.7	0.25	NS
Glutamic acid	2,381	2072	2,984	3,601	922.8	<0.01	NS
Glycine	957	776	1,062	1,206	306.7	0.04	NS
Proline	980	838	1,154	1,331	322.3	<0.01	NS
Serine	1,104	914	1,279	1,476	350.6	<0.01	NS
Tyrosine	618	504	722	832	220.5	0.01	NS
Total amino acid	19,227	15,728	22,007	25,458	6,585.8	0.01	NS

[1] Significant linear effects, $P < 0.05$.
[2] Interaction between location and diet was determined for 5 and 21 days
[2] Number of replicates. Each replicate is one cage and each cage represents an experimental unit with 30 birds per cage.
[3] Not significant, $P > 0.1$

Table 5. Ileal endogenous amino acids flow (mg/kg DM intake) in poults fed four levels of casein diets (d 15, mean for one experimental location)

	Dietary casein level, g/kg					
Item	0	50	100	150	SD[2]	Linear effect[1]
N[2]	6	5	4	5		
Essential amino acid flow, mg/kg DMI						
Arginine	274	300	383	359	49.1	<0.01
Histidine	138	160	218	216	28.9	<0.01
Isoleucine	263	393	624	669	95.2	<0.01
Leucine	414	483	668	643	89.7	<0.01
Lysine	276	328	486	529	106.9	<0.01
Methionine	86	121	165	171	23.0	<0.01
Phenylalanine	258	275	368	334	44.3	<0.01
Threonine	427	437	578	589	68.5	<0.01
Valine	409	493	712	736	93.7	<0.01
Nonessential amino acid flow, mg/kg DMI						
Alanine	307	356	500	497	72.2	<0.01
Aspartic acid	560	653	897	905	117.5	<0.01
Cysteine	211	227	284	245	21.3	<0.01
Glutamic acid	707	1,109	1,726	1,936	275.7	<0.01
Glycine	336	362	472	446	59.0	<0.01
Proline	426	453	611	612	57.7	<0.01
Serine	385	535	798	897	115.8	<0.01
Tyrosine	188	210	281	269	34.7	<0.01
Total amino acid	6,429	7,407	10,330	10,518	1,327.2	<0.01

[1] Significant linear effects, $P < 0.05$.
[2] Number of replicates. Each replicate is one cage and each cage represents an experimental unit with 10 birds per cage.

Table 6. Ileal endogenous amino acids flow (mg/kg DM intake) in poults fed four levels of casein diets (d 21 mean for two experimental locations)

	Dietary casein, g/kg						
Item	0	50	100	150	SD	Linear effect[1]	Location*diet[2]
N[3]	12	12	11	10			
Essential amino acid flow, mg/kg DMI							
Arginine	272	316	284	410	115.9	0.02	NS[4]
Histidine	158	187	182	263	63.3	<0.01	NS
Isoleucine	242	388	464	666	113.4	<0.01	NS
Leucine	408	501	480	716	172.1	<0.01	NS
Lysine	273	364	354	557	136.7	<0.01	NS
Methionine	90	127	122	180	43.8	<0.01	NS
Phenylalanine	263	293	263	388	101.7	0.02	NS
Threonine	454	504	502	706	141.4	<0.01	NS
Tryptophan	71	70	71	83	25.9	0.41	NS
Valine	389	495	542	773	139.2	<0.01	NS
Nonessential animo acid flow, mg/kg DMI							
Alanine	308	384	371	552	129.3	<0.01	NS
Aspartic acid	568	711	711	1,037	226.3	<0.01	NS
Cysteine	212	225	228	287	46.9	<0.01	NS
Glutamic acid	687	1,170	1,444	2,070	343.9	<0.01	NS
Glycine	324	365	356	491	110.7	<0.01	NS
Proline	381	458	505	712	116.9	<0.01	NS
Serine	401	604	751	1,012	143.1	<0.01	NS
Tyrosine	193	223	204	303	72.0	<0.01	NS
Total amino acid	6,843	8,094	8,463	12,078	2,326.6	<0.01	NS

[1] Significant linear effects, $P < 0.05$.
[2] Interaction between location and diet was determined using d 5 and d 21 data.
[3] Number of replicates. Each replicate is one cage and each cage represents an experimental unit with 8 birds per cage.
[4] Not significant, $P > 0.1$.

Running Title: ENDOGENOUS AMINO ACID FLOW IN CHICKS AND POULTS

Section: Metabolism and nutrition

Comparison of endogenous ileal amino acid and nitrogen flow in turkey poults and broiler chicks[1]

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ABSTRACT Ileal endogenous amino acid (IEAA) and nitrogen (N) flow in turkey poults and broiler chicks at three ages (5, 15, and 21 d) were compared by feeding a N-free diet (NFD) or graded levels of casein (CDP). The semi-purified diets contained 0 (NFD), 50, 100, or 150 g casein/kg diet as the only source of amino acids. Each diet was fed for 5 d prior to the collection of ileal digesta. Each diet was fed to six replicate cages containing 30 (5 d), 10 (15 d), or 8 (21 d) birds per cage. At d 5, IEAA and N flow (mg/kg DM intake) in poults fed the NFD, and graded levels of casein at d 5 was higher ($P < 0.05$) in poults than in chicks (NFD: Met, poult=391 and chick=153; Thr, poult=1,173 and chick=567; 50 g casein: Met, poult=339 and chick=276; Thr, poult=1,081 and chick=870: 100 g casein: Met, poult=472 and chick=346; Thr, poult=1,425 and chick=998; 150 g casein: Met, poult=539 and chick=399; Thr, poult=1,595 and chick=1,208). Within each species, there were differences ($P < 0.05$) in IEAA and N flow between 5 d and 21 d. The IEAA flow (mg/kg DM intake) in poults on d 15 (Met, 86; Thr, 427) and d 21 (Met, 85; Thr, 442) were not different from that of chicks fed the NFD on d 5 (Met, 153; Thr, 567). Similar trends were observed in the remaining three diets. An interaction between species and age was observed for most of the amino acids and N in birds fed NFD (Met, P=0.006, Thr, P=0.01), 50 g casein (Met, P=0.23; Thr, P=0.02), 100 g casein (Met, P=0.02, Thr, P=0.0002), and 150 g casein (Met, P=0.09; Thr, P=0.04). The results from this study suggest that at younger ages poults have significantly higher concentration of IEAA and N relative to chicks, however, by 15 and 21 d, the species differences in IEAA and N flow were not significant. The increased IEAA flow observed at the younger age should be taken into consideration when formulating starter diets on digestible protein basis.

Introduction

The goal of poultry nutrition is to optimize production through feeding diets that will meet the bird's requirements while minimizing cost of production and nutrient excretion. It has been advocated that poultry diet formulation should be based on digestible amino acid rather than total amino acid contents in the diets. In order to achieve this, it is important to quantify amino acids and nitrogen (N) of endogenous origin. This information can then be used to determine standardized ileal digestibility coefficients of feed ingredients. A number of methods have been used to determine this estimate. This includes feeding of nitrogen-free diet (NFD), feeding of completely digestible protein (CDP), and the use of regression method. Other methods include the use of guanidinated casein and homoarginine method. However, each of these methods has its own limitations. The review by Nyachoti et al. (1997) on pigs examined the merits and demerits of each of the methods. Estimates from any of these methods can be used to standardized digestibility values by serving as a correction factor.

The origin of IEAA varies and it includes protein from desquamated epithelial cells lining the gastro intestinal tracts (GIT), serum albumin, mucoprotein and the various digestive secretions (Moughan et al., 1992a; Nyachoti et al., 1997). The effects of age of birds and the class of poultry on IEAA flow have been reported (Ravindran and Hendriks, 2004, Ravindran et al., 2004). Endogenous amino acid flow in chickens has been reported **to increase** with age. However, there is a dearth of information on IEAA flow in chicks and turkey poults at very early ages, neither is there any study that compares IEAA or N flow in these species.

In this study we determined and compared IEAA flow in broiler chicks and turkey poults at three ages (d 5, 15, and 21) using two different methods of endogenous secretion estimation. The methods employed were feeding of NFD and feeding of CDP. The hypothesis tested was that the IEAA flow is dependent on species, age, and method of determination. The objectives of the study were to determine the effects of age and method of determining IEAA flow on IEAA flow in chicks and poults and to compare species effect.

Material and Methods

Diet formulation

Four semi purified diets were used in this study. The diets included a nitrogen-free diet (NFD, 0 g casein/kg diet), diet containing 50, 100, or 150 g casein/kg diet (Table 1). Casein supplied all the amino acids in the diets. Two basal diets (a conventional broiler starter diet and a turkey starter diet) were also fed. The starter diets were fed to the respective species prior to the time the treatment diets were fed. The basal diets were formulated to meet the NRC (1994) recommendation. Chromium, which was used as an indigestible marker, was added to the treatment diets at 3 g/kg diet.

Birds and housing

One thousand one hundred and fifty two 1-d old male broiler chicks (ROSS) and 1,152 male turkey poults **(???????)** were obtained from commercial hatcheries. Seven hundred and twenty birds from each species were weighed individually and randomly allocated to diets on d 0.

Each diet was fed for 5 d before ileal contents collected. Six replicate cages containing 30 birds per cage were euthanized and the ileal contents removed by flushing with distilled water on d 5. The remaining birds were fed the conventional corn and soybean meal-based diet appropriate for the respective species until day 10 when 240 birds were randomized to cages with 10 birds per cage and 6 replicate cages per diet. These birds were euthanized and the ileal contents collected on day 15. On day 16, 192 birds were placed on the experimental diets and were euthanized and ileal contents collected on day 21.

Birds were raised in battery cages (Alternative design) till the end of the study in an environmentally controlled room with 24-h of light. Room temperature was 35°C during the first week. The temperature was dropped by 5 degrees during the subsequent weeks. Birds had free access to water. All animal care procedures were approved by the Purdue University Animal Care and Use Committee.

Sampling and ileal digesta processing

On d 5, 15, and 21 after the birds had been euthanized, content from the ileal (portion of the small intestine from Meckel's diverticulum to about 5 mm proximal to the ileo-cecal junction) region was flushed with distilled water. For birds sampled on d 5, **50 ml** syringe was used for flushing while wash bottle was used on d 15 and 21. Ileal digesta from birds within a cage was pooled. Ileal digesta were stored in a freezer (-40°C) until they were processed. Samples were freeze-dried, ground using mortar and pestle, and were sent to the University of Missouri Experiment Station Chemical Laboratory for N, complete amino acid profile, and chromium analyses.

Chemical analysis

Dry matter content was determined on ground diets and ileal digesta by drying the samples at 100°C for 24 h. Amino acids and chromium analyses were conducted at the University of Missouri Experiment Station Chemical Laboratory. For amino acid analyses, samples were hydrolyzed in a 6 *N* HCl for 24 h at 110°C under N atmosphere. For sulfur containing amino acids, methionine and cysteine, performic acid oxidation was carried out before acid hydrolysis. Samples for tryptophan analysis were hydrolyzed using barium hydroxide. The amino acids in the hydrolysate were then determined by HPLC after postcolumn derivatization (AOAC, 2000; 982.30 E [a, b, c]. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Chromium was determined by the inductively coupled plasma atomic emission spectroscopy method (AOAC, 2000; 990.08) following nitric/perchloric acids wet ash digestion. Nitrogen in both the diets and ileal digesta was determined by the combustion method (AOAC, 2000; 990.03) (model FP2000, LECO Corp., St. Joseph, MI) using EDTA as a standard.

Calculations

Ileal EAA and N flow from both species was calculated as mg of N or amino acid flow per 1 kg of feed intake on DM using the following formula by Moughan et al. (1992b):

Amino acid flow (mg/kg DM intake) = [amino acid in ileal digesta, mg/kg] x ((diet chromium, mg/kg)/(ileal chromium, mg/kg))

Statistical analysis

The data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with species and age as the class variables. Where F-ratios indicate significance, treatment means were separated using Duncan multiple range test. Level of significance was set at $P < 0.05$.

Results

The analyzed dietary amino acid and N contents (on DM basis) are reported in Table 2. Ileal EAA flow in chicks and poults at three ages when fed NFD are reported in Table 3. The IEAA flow at d 5 was higher ($P < 0.05$) in poults than in chicks. Percent N flow in chicks on d 5, 15, and 21 were about 46, 66, and 60% of N flow in poults at the same age (chick vs. poult; d 5: 9,045 vs. 19,814; d 15: 4,242 vs. 6,429; d 21: 3,935 vs. 6,610 mg/kg DMI). Glutamic acid, aspartic cid, and leucine were the amino acids with the greatest flow in both species on d 5 (poults vs. chicks: glutamic acid 2,489 vs. 1,044; aspartic acid 1,923 vs. 840; leucine 1,639 vs. 667 mg/kg DMI). On d 15 and d 21 glutamic acid, aspartic acid and threonine had the greatest flow. Methionine and threonine flow in chicks on d 5, 15, and 21 were, respectively, 39 and 48%, 65 and 70%, and 52 and 59% of endogenous methionine and threonine flow in poults at the same age. There was no significant difference in nitrogen flow between the two species on d 15 and d 21. Similar trends were seen in all the amino acids as well. There was an interaction between age and species for all the amino acids when NFD was fed.

Feeding of diet containing 50g casein/kg diet (Table 4), resulted in significant differences in IEAA flow except for isoleucine, lysine, tryptophan, glutamic acid, proline, serine, tyrosine, and N between the two species on d 5. However, IEAA and N flow were higher ($P < 0.05$) on d 5 relative to flow on d 15 and d 21. Endogenous N flow in chicks on d 5, 15, and 21 were 89, 99, and 89% of N flow in poults of the same age. Endogenous amino acid and N flow on d 15 and 21 were not significantly different between the two species. Glutamic acid, aspartic acid, leucine and threonine were the amino acids with the greatest flow at the three ages evaluated. Methionine and threonine flow in chicks on d 5, 15, and 21 were, respectively, 81 and 80%, 94 and 108%, and 85 and 90% of endogenous methionine and threonine flow in poults of the same age. Significant interaction between species and age were seen for EAA flow of threonine, valine, alanine, cysteine, glycine, and tyrosine.

Endogenous amino acids and N flow in broiler chicks and turkey poults when diet containing 100 g casein/kg diet was fed are reported in Table 5. Endogenous amino acid (except glutamic acid) and N was higher ($P < 0.05$) in poults than in chicks on d 5. Chicks' endogenous methionine, threonine and N flow was 73, 70, and 74% that of poults. Endogenous amino acid (except for cysteine and glycine), and N flow was not different (d 15) with chicks' endogenous methionine, threonine, and N flows being 78, 94, and 86% that of poults at the same age. On d 21, IEAA and N flow were not significantly different between the two species. However, endogenous methionine, threonine, and N flows were 115, 102, and 107% of the respective flows in poults. The IEAA and N flows in the two species were higher ($P < 0.05$) on d 5 when compared with flows on d 15 and d 21. Except for glutamic acid, interaction between species and age were significant ($P < 0.05$).

When diet containing 150 g casein/kg diet was fed to poults and chicks (Table 6), IEAA (except for isoleucine, lysine, tryptophan, glutamic acid, proline, serine, and tyrosine) and N flows were higher $(P < 0.05)$ in poults (d 5) while flows were not significantly different between the two species on d 15 and d 21. Endogenous methionine and threonine flows in chicks on d 5, 15, and 21 were, respectively, 74 and 76%, 108 and 121%, and 122 and 102% of endogenous methionine and threonine flow in poults of the same age. Significant interaction between species and age were seen for amino acids threonine, alanine, aspartic acid, cysteine, glycine, and N. Nitrogen flow in chicks was 80, 118, and 106% that of poults, for d 5, 15, and 21, respectively.

Discussion

The objectives of this study were to determine and compare the effects of age and method of IEAA and N flow determination in chicks and poults and to determine if there is species by age interaction on IEAA and N flow. Same diets were fed to both species and were made from the same batch of ingredients. Birds were in good condition of health throughout the duration of the study and mortality was less than 2.5% for each of the treatments. Mean body weight at sampling (NFD) was chick, 47 g, poult, 57 g (d 5); chick, 180 g, poult, 168 g (d 15); chick, 312 g, poult, 318 g (d 21). When 50 g casein/kg diet was fed, mean body weight at sampling (chick vs. poult) at d 5, 15, and 21 were 53 vs. 61 g, 192 vs. 167 g, 318 vs. 327 g, respectively.

Mean body weight at sampling when 100 g casein/kg diet was fed (chick vs. poult) were 58 vs. 64 (d 5), 203 vs. 180 (d 15), and 180 vs. 343 (d 21). The mean weight for birds on 150 g casein/kg diet at sampling were 63 vs. 65 (d 5), 225 vs. 198 (d 15), and 363 vs. 362 (d 21) for chick and poult, respectively.

According to Mitchell (1924), endogenous N losses are the N found in digesta or in feces of animals fed NFD. A number of studies have been conducted to determine the IEAA and N flow in chicks and chickens (Ravindran and Hendrikcs, 2004; Ravindran et al., 2004). However, to the best of the authors' knowledge no such study have been conducted in 5-d old broiler chicks and turkey poults and no comparison in IEAA flow between the two species have been made. In most of the studies available, IEAA and N flow determination was conducted in older chickens e.g. 2, 4 or 5 week old broiler chickens (Ravindran et al., 2004), 6 wk old broiler, 70 wk old layers, and 70 wk old rooster (Ravindran and Hendriks, 2004). In addition to this, methods of estimation used include feeding of NFD, guanidinated casein (GuC), and enzyme hydrolyzed casein (EHC) (Ravindran et al., 2004), peptide alimentation method (Ravindran and Hendriks, 2004). In this study we determined IEAA and N flow at three different ages (d 5, 15, and 21) and in two poultry species (broiler chicks and turkey poults) using two methods (NFD and feeding of CDP methods).

Level of feed intake, especially the level of protein intake has been reported in growing pig and rat to be positively correlated with endogenous amino acid flow (Darragh et al., 1990; Butts et al., 1993) by increasing endogenous secretion. Also the presence of protein in the gut that are of dietary origin may negatively impact breakdown and re-absorption of protein of endogenous origin (Snook and Meyer, 1964). It is also expected that body size should play a factor as heavier birds are expected to have higher intake coupled with heavier GIT could result in increase surface area. Increased surface area could lead to increased IEAA flow due to increased rate of sloughing off of intestinal cells. In this study, poults' d 5 final body weight was 6 g higher than that of chicks. On d 15 chicks were 22 g heavier than poults while poults were 3 g heavier than chicks at d 21.

Results when NFD was fed shows that ileal methionine, threonine, and N flow in chicks were about 39, 48, and 46%; 65, 70, and 65%; or 53, 59, and 60% of their respective flow in poults on d 5, 15, or 21. This result shows that irrespective of the body weight at sampling poults had higher level of IEAA and N flow than chicks when fed NFD. Despite the fact that poults were about 10 g (21%) or 6 g (2%) heavier than chicks on d 5 and 21, respectively, and that poults consumed more feed than poults at sampling (d 21), it is difficult to attribute the difference, especially on d 5 to either weight or feed intake alone.

Increase in the level of dietary protein by the inclusion of casein (from 0 to 150 g casein/kg diet) resulted in increase in IEAA and N flow at the three ages investigated and in both species. However, in poults where diet containing 50 g casein resulted in relatively lower IEAA and N flow on d 5. This diet (50 g casein at age d 5) also resulted in a relative increase in the level of IEAA and N flow in chicks with methionine, threonine, and N flow in chicks being, respectively, about 81, 80, and 89% of that of poults. Unlike what was observed with NFD, 100, or 150 g casein diets, there was no significant difference in flow between the two species on d 5 when diet containing 50 g casein/kg diet was fed. For all the four levels of casein in the diets, there was no difference in IEAA and N flow between d 15 and 21 and flow at these ages were significantly lower that at their respective d 5.

The large increase in IEAA flow in chicks when diet containing graded levels of casein was fed could be an indication that chicks respond to the stimulatory effects of dietary protein on digestive tract for increased endogenous secretion or increase in the rate of cell turnover in poults. For instance, when 50 g casein/kg diet was fed, the proportion of chick to poult N flow increased for d 5, 15, or 21 from 46, 66, or 60% (NFD) to 89, 99, or 89% (50 g casein diet/kg diet), respectively. This proportion is similar in almost all the amino acids (lysine, methionine, and threonine: NFD, 45, 39, or 48%; 50 g casein diet, 97, 81, or 80%) on d 5. Glutamic acid flow, although, very high did not differ between chicks and poults on d 5 when 50, 100, or 150 g casein/kg diet was fed within each of the three age evaluated. The high proportion of glutamic acid , aspartic acid, threonine, serine, leucine, and lysine may be as a result of slow re-absorption from the gut as compared to other amino acids (Taverner et al. 1981). In addition to this, isoleucine, lysine, proline, serine, and tyrosine flows were not different on d 5 when diets containing either 50 or 150 g casein were fed. The greater increase in IEAA and N flow when protein containing diets were fed could be attributed to either an increase in digestive secretion in chicks or a decrease in the rate of digestion and absorption of EAA and N relative to poults or the other way round. The decrease in IEAA and N flow with age (d 5 vs. d 15 or d 21) could be attributed to an improvement in the digestion and re-absorption of amino acids that are of endogenous origin after the first week. The use of EHC in determining IEAA flow when compared to NFD method resulted in higher (2 to 3 folds increase) values of IEAA (Kadim et al., 2002; Ravindran and Hendriks, 2004).

Free amino acids and small peptides have been reported to be readily absorbed in pigs leaving the bulk of IEAA and N flow to be composed of mucin proteins due to their resistance to enzymatic hydrolysis (Moughan and Schuttert, 1991). Based on the this report, it can be said that at d 5 turkeys poults produced a higher amount of mucin relative to chicks as is evident whether IEAA flow determination was by either NFD or CDP. When fed NFD the level of lysine in the digesta of chicks was about half of the poults, however this difference was greatly reduces when CDP was fed especially diet containing 50 g casein.

The results from this study shows that IEAA and N flow is age, species, and method dependent. More IEAA and N were seen at younger age (d 5) in both species relative to d 15 and 21. Turkey poults produced higher IEAA and N relative to broiler chicks on d 5, however, the difference between the two species disappeared between d 15 and 21. Also, the relative level of IEAA and N flow is a function of the method used. Flow increased linearly with increasing level of dietary protein. Finally, it can be concluded that correction for endogenous secretion is needed especially at younger ages and that the effects of dietary protein on IEAA and N flow is more pronounced in young chicks than poults.

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Table 1. Dietary composition of experimental diets (g/kg, on as fed basis)

Ingredient g/kg	N-F-D	50 g casein	100 g casein	150 g casein
Corn starch	169.0	119.0	69.0	19.0
Dextrose	640.0	640.0	640.0	640.0
Casein	0.0	50.0	100.0	150.0
Solkafloc	50.0	50.0	50.0	50.0
Soyabean oil	50.0	50.0	50.0	50.0
Vitamin-Mineral Premix[1]	5.0	5.0	5.0	5.0
Dicalcium phosphate	19.0	19.0	19.0	19.0
$NaHCO_3$	20.0	20.0	20.0	20.0
KCl	12.0	12.0	12.0	12.0
MgO	2.0	2.0	2.0	2.0
Choline chloride	3.0	3.0	3.0	3.0
Limestone	13.0	13.0	13.0	13.0
NaCl	2.0	2.0	2.0	2.0
Chromic Ox Premix[2]	15.0	15.0	15.0	15.0
Total	1000.0	1000.0	1000.0	1000.0

[1]Provided per kg of diet: Vitamin A (retinyl acetate), 7,320 IU; Vitamin D_3 (cholecalciferol) , 729 IU; Vitamin E (DL-α-tocopheryl acetate), 27.9 IU; Vitamin K activity, 5.7 mg; Menadione, 1,800 μg; Vitamin B_{12}, 37.2 mg; Riboflavin, 7.2 mg; D-Pantothenic acid, 27 mg; Niacin, 42 mg.

[2]Chromic oxide (Cr_2O_3) premix added as index at a ratio 1:4 of chromic oxide:corn starch.

Table 2. Dietary composition of nitrogen free diet, and diets containing graded levels of casein (g/kg DM basis)[1]

	Dietary casein level, g/kg			
	0 (NFD)[2]	50	100	150
Item				
Essential amino acids				
Arginine	0.00	1.41	3.15	5.00
Histidine	0.00	1.19	2.61	4.13
Isoleucine	0.00	2.17	4.56	7.28
Leucine	0.00	4.12	8.58	13.69
Lysine	0.20	3.47	7.38	12.06
Methionine	0.00	0.98	2.28	3.69
Phenylalanine	0.00	2.06	4.45	7.61
Threonine	0.00	1.63	3.47	5.54
Tryptophan	<0.40	0.65	1.11	1.74
Valine	0.00	2.82	5.97	9.45
Nonessential amino acids				
Alanine	0.00	1.30	2.71	4.35
Aspartic acid	0.00	3.04	6.40	10.21
Cysteine	0.00	0.22	0.43	0.65
Glutamic acid	0.10	9.55	19.97	31.73
Glycine	0.00	0.76	1.63	2.61
Proline	0.00	0.45	0.96	1.48
Serine	0.00	2.17	4.34	6.84
Tyrosine	0.00	1.41	3.91	6.74
Nitrogen	1.52	44.48	93.46	148.96

[1]Analyzed dietary amino acid components of experimental diets, g/kg diet
[2]Nitrogen-free diet

Table 3. Ileal endogenous amino acids and nitrogen flow (mg/kg DM intake) in poults & chicks fed nitrogen free diet

	Age, days[1]							
	5		15		21			
	Poults	Chicks	Poults	Chicks	Poults	Chicks	SD[2]	Spe*Age
N	5	5	6	6	6	5		
Essential amino acid flow, mg/kg DMI[3]								
Arginine	1,017.8^{a}	462.6^{b}	273.7^{bc}	178.5^{c}	270.8^{bc}	159.2^{c}	185.366	0.0159
Histidine	520.6^{a}	190.0^{b}	138.0^{b}	80.8^{b}	141.5^{b}	70.0^{b}	96.151	0.0058
Isoleucine	917.0^{a}	389.8^{b}	263.5^{bc}	174.3^{c}	240.7^{bc}	158.8^{c}	154.209	0.0045
Leucine	1,638.6^{a}	667.0^{b}	414.2^{bc}	276.0^{c}	403.8^{bc}	241.8^{c}	286.698	0.0043
Lysine	1,168.2^{a}	522.4^{b}	276.2^{bc}	208.7^{c}	286.5^{bc}	182.0^{c}	232.796	0.0175
Methionine	390.6^{a}	153.4^{b}	86.0^{bc}	56.0^{bc}	84.5^{bc}	44.4^{c}	72.516	0.0055
Phenylalanine	964.6^{a}	407.4^{b}	258.0^{bc}	182.8^{c}	256.0^{bc}	152.8^{c}	164.561	0.0045
Threonine	1,173.0^{a}	566.8^{b}	426.5^{bc}	297.2^{c}	442.2^{bc}	262.8^{c}	176.097	0.0103
Valine	1,357.8^{a}	516.6^{b}	408.7^{bc}	232.0^{c}	421.8^{bc}	205.4^{c}	210.534	0.0023
Nonessential amino acid flow, mg/kg DMI								
Alanine	1,152.8^{a}	450.0^{b}	307.3^{bc}	186.0^{bc}	298.0^{bc}	167.4^{c}	203.039	<0.0001
Aspartic acid	1,923.0^{a}	840.8^{b}	560.0^{bc}	381.7^{c}	541.0^{bc}	328.6^{c}	322.503	0.0062
Cysteine	549.8^{a}	234.2^{b}	211.0^{bc}	132.0^{c}	227.3^{b}	138.0^{c}	66.650	0.0006
Glutamic acid	2,489.0^{a}	1,044.2^{b}	706.8^{bc}	430.0^{b}	659.2^{b}	430.6^{b}	448.398	0.0086
Glycine	1,004.2^{a}	433.4^{b}	336.0^{bc}	218.7^{bc}	321.8^{bc}	195.0^{c}	167.820	0.0078
Proline	1,039.8^{a}	450.6^{b}	425.7^{bc}	249.7^{c}	417.2^{b}	245.4^{b}	155.656	<0.0001
Serine	1,114.2^{a}	537.4^{b}	385.3^{bc}	261.3^{c}	398.0^{bc}	268.0^{c}	171.337	0.0089
Tyrosine	633.8^{a}	295.0^{b}	187.7^{bc}	143.8^{c}	191.8^{bc}	129.6^{c}	109.571	0.0091
Nitrogen	19,813.8^{a}	9,045.4^{b}	6,429.0^{bc}	4,242.0^{c}	6,609.8^{bc}	3,934.6^{c}	3,317.017	0.0124

[1] Number of replictes. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).
[2] Standard deviation. [3] Means within the same row with different superscripts are significantly different, $P < 0.05$.

Table 4. Ileal endogenous amino acids and nitrogen flow (mg/kg DM intake) in poults & chicks fed diet containing 50 g casein/kg diet

	Age, days[1]							
	5		15		21			
	Poults	Chicks	Poults	Chicks	Poults	Chicks	SD[2]	Spe*Age
N	5	5	5	5	6	5		
Essential amino acid flow, mg/kg DMI[3]								
Arginine	854.8^{a}	706.4^{b}	300.2^{c}	262.4^{c}	270.0^{c}	222.2^{c}	83.026	0.2721
Histidine	431.4^{a}	333.2^{b}	160.0^{c}	137.6^{c}	152.8^{c}	116.8^{c}	38.379	0.0777
Isoleucine	846.0^{a}	791.6^{a}	392.6^{b}	388.2^{b}	372.0^{b}	354.2^{b}	83.379	0.7870
Leucine	$1,427.0^{a}$	$1,131.2^{b}$	482.8^{c}	422.4^{c}	436.7^{c}	358.4^{c}	133.358	0.1075
Lysine	988.2^{a}	955.4^{a}	328.4^{b}	378.8^{b}	318.2^{b}	307.4^{b}	104.425	0.9522
Methionine	338.8^{a}	275.8^{b}	120.6^{c}	113.2^{c}	107.0^{c}	91.2^{c}	37.676	0.2229
Phenylalanine	830.4^{a}	656.8^{b}	274.6^{c}	242.2^{c}	246.3^{c}	202.8^{c}	77.041	0.0923
Threonine	$1,080.6^{a}$	869.8^{b}	437.0^{c}	474.0^{c}	404.7^{c}	364.8^{c}	92.326	0.0181
Tryptophan	116.8^{a}	80.80^{ab}			52.0^{b}		23.699	
Valine	$1,257.8^{a}$	932.4^{b}	492.8^{c}	422.6^{c}	462.0^{c}	372.8^{c}	108.045	0.0234
Nonessential amino acid flow, mg/kg DMI								
Alanine	$1,027.8^{a}$	701.8^{b}	356.4^{c}	310.6^{c}	317.3^{c}	262.8^{c}	89.462	0.0020
Aspartic acid	$1,729.2^{a}$	$1,399.2^{b}$	653.2^{c}	660.0^{c}	591.2^{c}	535.2^{c}	158.39	0.0571
Cysteine	471.4^{a}	296.4^{b}	226.6^{c}	180.2^{c}	210.0^{c}	191.6^{c}	33.927	<0.0001
Glutamic acid	$2,419.2^{a}$	$2,496.2^{a}$	$1,108.6^{b}$	$1,152.4^{b}$	$1,065.2^{b}$	$1,044.4^{b}$	260.619	0.9090
Glycine	878.8^{a}	624.6^{b}	361.8^{c}	316.8^{c}	317.8^{c}	279.4^{c}	70.908	0.0026
Proline	991.6^{a}	906.8^{a}	453.4^{b}	435.6^{b}	436.2^{b}	380.6^{b}	88.402	0.7005
Serine	$1,048.6^{a}$	961.6^{a}	535.2^{b}	592.2^{b}	538.5^{b}	546.4^{b}	102.134	0.2938
Tyrosine	564.6^{a}	533.2^{a}	210.2^{b}	205.4^{b}	192.5^{b}	172.0^{b}	60.683	<0.0001
Nitrogen	$17,719.8^{a}$	$15,732.0^{a}$	$7,407.4^{b}$	$7,367.4^{b}$	$7,215.5^{b}$	$6,399.8^{b}$	1,654.544	0.4277

[1]Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).
[2]Standard deviation.
[3] Means within the same row with different superscripts are significantly different, $P < 0.05$.

Table 5. Ileal endogenous amino acids and nitrogen flow (mg/kg DM intake) in poults & chicks fed diet containing 100 g casein/kg diet

	Age, days[1]							
	5		15		21			
	Poults	Chicks	Poults	Chicks	Poults	Chicks	SD[2]	Spe*Age
N	4	6	4	4	6	6		
Essential amino acid flow, mg/kg DMI[3]								
Arginine	1,152.5^{a}	774.0^{b}	383.0^{c}	305.0^{c}	271.8^{c}	291.0^{c}	108.421	0.0010
Histidine	588.5^{a}	399.8^{b}	217.8^{c}	163.5^{c}	167.7^{c}	168.8^{c}	47.587	0.0004
Isoleucine	1,206.0^{a}	938.5^{b}	623.8^{c}	528.0^{c}	497.5^{c}	542.5^{c}	118.365	0.0196
Leucine	1,920.0^{a}	1,287.2^{b}	667.8^{c}	500.8^{c}	479.2^{c}	509.0^{c}	174.261	0.0007
Lysine	1,472.5^{a}	1,145.2^{b}	485.5^{c}	454.0^{c}	349.2^{c}	414.3^{c}	149.966	0.0181
Methionine	471.8^{a}	345.5^{b}	165.0^{c}	129.5^{c}	126.2^{c}	145.5^{c}	55.249	0.0196
Phenylalanine	1,109.3^{a}	737.8^{b}	367.8^{c}	273.8^{c}	247.3^{c}	267.3^{c}	105.090	0.0009
Threonine	1,425.0^{a}	998.2^{b}	578.0^{c}	541.8^{c}	461.7^{c}	469.7^{c}	108.789	0.0002
Tryptophan	146.5^{a}	92.8^{ab}		70.0^{b}			31.181	
Valine	1,667.3^{a}	1,091.0^{b}	712.3^{c}	574.0^{c}	567.8^{c}	536.3^{c}	136.532	0.0003
Nonessential amino acid flow, mg/kg DMI								
Alanine	1,387.8^{a}	802.5^{b}	499.5^{c}	372.0^{c}	353.7^{c}	361.2^{c}	117.922	<0.0001
Aspartic acid	2,339.8^{a}	1,603.8^{b}	896.5^{c}	777.0^{c}	678.5^{c}	745.5^{c}	194.437	0.0003
Cysteine	582.8^{a}	314.5^{b}	283.8^{b}	193.5^{c}	224.8^{c}	230.7^{c}	39.585	<0.0001
Glutamic acid	3,531.0^{a}	3,096.3^{a}	1,725.8^{b}	1,621.3^{b}	1,477.2^{b}	1,648.7^{b}	337.882	0.1389
Glycine	1,182.3^{a}	683.7^{b}	471.8^{c}	348.3^{d}	344.7^{d}	354.8^{d}	86.694	<0.0001
Proline	1,330.8^{a}	1,096.0^{b}	611.0^{c}	501.0^{c}	513.2^{c}	529.8^{c}	94.864	0.0189
Serine	1,439.0^{a}	1,155.3^{b}	797.5^{c}	789.5^{c}	747.0^{c}	832.3^{c}	143.667	0.0207
Tyrosine	784.0^{a}	601.0^{b}	281.3^{c}	224.0^{c}	203.2^{c}	235.3^{c}	73.549	0.0094
Nitrogen	24,381.3^{a}	18,152.3^{b}	10,330.0^{c}	8,883.5^{c}	8,304.5^{c}	8,893.5^{c}	2,056.835	0.0030

[1]Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).
[2]Standard deviation.
[3] Means within the same row with different superscripts are significantly different, $P < 0.05$.

Table 6. Ileal endogenous amino acids and nitrogen flow (mg/kg DM intake) in poults & chicks fed diet containing 150 g casein/ kg diet

	Age, days[1]							
	5		15		21			
	Poults	Chicks	Poults	Chicks	Poults	Chicks	SD[2]	Spe*Age
N	3	6	5	4	5	5		
Essential amino acid flow, mg/kg DMI[3]								
Arginine	1,290.7[a]	888.5[b]	359.4[c]	356.3[c]	371.6[c]	340.2[c]	178.274	0.0569
Histidine	668.0[a]	495.2[b]	216.0[c]	228.0[c]	232.2[c]	221.8[c]	89.646	0.0929
Isoleucine	1,387.7[a]	1,166.7[a]	669.4[b]	787.3[b]	682.6[b]	759.0[b]	203.356	0.2005
Leucine	2,169.0[a]	1,536.0[b]	643.4[c]	637.5[c]	678.2[c]	645.6[c]	302.128	0.0765
Lysine	1,617.0[a]	1,402.3[a]	529.4[b]	678.0[b]	529.2[b]	625.4[b]	244.670	0.2804
Methionine	539.3[a]	399.0[b]	171.2[c]	184.3[c]	170.6[c]	208.4[c]	85.266	0.0874
Phenylalanine	1,235.7[a]	861.7[b]	334.0[c]	316.0[c]	351.2[c]	321.6[c]	172.672	0.0771
Threonine	1,595.3[a]	1,208.2[b]	589.2[c]	712.8[c]	643.6[c]	653.2[c]	200.965	0.0407
Tryptophan	167.0[a]	115[ab] (6)	.	63.0[b]	62.5[b]	73.5[b]	28.446	0.0979
Valine	1,896.0[a]	1,354.0[b]	736.2[c]	778.3[c]	778.8[b]	741.0[c]	252.406	0.0560
Nonessential amino acid flow, mg/kg DMI								
Alanine	1,513.7[a]	952.5[b]	497.2[c]	500.8[c]	511.4[c]	475.8[c]	190.646	0.0136
Aspartic acid	2,640.3[a]	1,953.0[b]	904.6[c]	1,077.8[c]	949.8[c]	1,019.8[c]	346.951	0.0371
Cysteine	633.7[a]	350.0[b]	254.2[b]	231.3[b]	293.2[b]	250.4[b]	81.396	<0.0001
Glutamic acid	4,136.7[a]	3,930.2[a]	1,936.2[b]	2,561.5[b]	1,994.0[b]	2,491.6[b]	608.625	0.3400
Glycine	1,308.7[a]	829.3[b]	446.2[c]	431.3[c]	456.8[c]	425.4[c]	150.119	0.0061
Proline	1,483.3[a]	1,459.8[a]	612.4[b]	672.5[b]	691.6[b]	729.2[b]	195.269	0.9030
Serine	1,688.0[a]	1,387.7[ab]	897.0[c]	1,193.0[bc]	973.4[c]	1,143.8[c]	247.633	0.0530
Tyrosine	880.0[a]	714.3[a]	269.4[b]	284.5[b]	287.2[b]	300.6[b]	129.858	0.2804
Nitrogen	27,495.7[a]	21,904.2[b]	10,518.4[c]	12,446.0[c]	11,477.2[c]	12,181.2[c]	3,696.984	<0.0001

[1]Number of replicates. Each cage represents an experimental unit with 30 birds per cage (day 5), 10 birds per cage (day 15), and 8 birds per cage (day 21).
[2]Standard deviation.
[3] Means within the same row with different superscripts are significantly different, $P < 0.05$.

Standardized ileal amino acid digestibility of some feed ingredients of plant sources in broiler chicks and turkey poults using a nitrogen-free or casein diet[1]

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STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY

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Abstract 1. The aim of this study was to determine apparent and standardized ileal amino acid, total amino acid, and nitrogen digestibility of 5 feed ingredients in 5- and 21-d old broiler chicks and turkey poults. Two methods of standardization, feeding of nitrogen-free diet and completely digestible protein, CDP, (10% casein), casein, were used.

2. The feed ingredients were highly and poorly digestible distillers dried grain soluble (HD and PD DDGS), canola meal, maize, and soybean meal. These ingredients were the sole source of amino acids in the diets. Diets, in mash form, and water were offered ad libitum with each diet containing 20% crude protein. Chromium, used as the indigestible marker, was added to each of the diets at the rate of 3 g/kg diet.

3. There were 6 replicates per diet with 30 and 8 birds per replicate which were sampled on d 5 and 21, respectively.

4. The results from these studies show that age of broiler and poults have significant effect on amino acid and nitrogen apparent digestibility. However, apparent digestibility for maize in chicks did not show any age effects except for histidine. The lowest and highest total amino acid digestibility for the two species were in maize and soybean meal, respectively.

5. In poults, both standardization methods resulted in improved standardized ileal amino acid digestibility (SIAAD), however, there was no significant difference between the two methods at a particular age.

6. For the chicks, both standardization methods resulted in improved SIAAD, however, unlike in the poults standardization using CDP resulted in higher ($P < 0.05$) SIAAD for TAA, N, and some of the amino acids on d 21 for HD DDGS, canola meal, maize, and SBM.

7. In summary, both the apparent and SIAAD was higher for the HD DDGS relative to the PD DDGS in both species. The SIAAD for canola meal and SBM were higher when compared to that of maize and DDGS.

8. This result shows that either of the two methods of standardization will produce similar results in poults at d 5 and 21 while chicks SIAAD is sensitive to method of standardization at d 21.

INTRODUCTION

Poultry productivity is based on accurate diet formulation to meet the maintenance and production requirements of the bird. In addition to meeting the bird's requirements, the need to reduce excess dietary amino acids thereby reducing nutrient excretion will result in increased profit margin and a reduction in environmental pollution from nutrient excretion.

In order to achieve this, a number of procedures to determining amino acid and nitrogen availability in feed ingredients have been developed. These procedures include *in vitro* and *in vivo* methods. The advantages of the *in vitro* methods include the fact that they are simple, rapid and easily reproducible (Ravindran and Bryden, 1999). The *in vivo* methods are the preferred method because such measurements are made under physiological conditions as opposed to trying to stimulate such as seen *in vitro* techniques. The *in vivo* methods include excreta (total tract) developed by Kuiken and Lyman (1948) or ileal apparent digestibility determination. The ileal method is preferred to the total tract method because it precludes the effects of the hind gut microbe on the digesta as sampling is done before the hindgut microflora are able to modify the amino acid in the digesta. Also, the complication arising from the mixing of the digesta and amino acids from urine is avoided. In addition to this, the effect of age and methods of apparent amino acid and nitrogen digestibility estimation has been determined in some strains of poultry (Kadim, et al., 2002; Huang, et al., 2005).

Despite the progress that has been made over the years to improve on quantitative measuring of amino acids digestibility, there has been concerted efforts to quantify the contribution of amino acid and nitrogen of endogenous origin to ileal digesta (Siriwan, et al., 1993;Ravindran et al., 2004; Ravindran and Hendriks, 2004) with the view of using this to determine the standardized ileal amino acid digestibility

(SIAAD). By correcting for the basal endogenous amino acid flow at the terminal ileum, it will be easy to determine the SIAAD of various feed ingredients thereby making feed formulation on digestible amino acid a lot more practical. The advantages of formulating broiler diets based on total digestible amino acids and undigestible crude protein is outline by de Lange et al. (2003) and Lemme et al., 2004.

The objective of this study, therefore, was to determine the apparent and SIAAD in 5- and 21-d old broiler chicks and turkey poults fed semi-purified diets containing amino acids from four plant ingredients using the basal endogenous amino acids from birds fed a nitrogen-free diet and completely digestible protein, CDP (10% casein) as the correction factors.

MATERIAL AND METHODS

Plant sources of amino acids

Four different plant sources of amino acids were used in this study. These feed ingredients include a highly and poorly digestible distillers dried grain solubles (HD and PD DDGS), canola meal, maize, and soybean meal.

Diet formulation

Two semi purified diets were used as the control diets to determine the basal endogenous amino acid flow. These diets were used for the standardization of the apparent digestibility values to arrive at the standardized ileal amino acid digestibility (SIAAD). The control diets were a NFD and a diet containing completely digestible protein source, CDP, (100 g casein/kg diet). The remaining five diets were formulated to contain about 20 % crude protein (CP) with each of the five plant ingredients supplying all the CP contents of the diets. Diet composition and analyzed dietary nutrient composition are reported in Tables 1 and 2, respectively. Two basal diets (a broiler starter and a turkey starter diet) were also made. Chromic oxide was added as the indigestible marker at 3 g/kg diet. Broiler chicks and turkey poults were fed the same experimental diets but the studies were conducted at two different locations.

Birds and housing

One thousand five hundred and ninety six 0-d old male broiler chicks (ROSS 308, Aviagen, Huntsville, AL) and 1,596 male turkey poults (Nicholas) were obtained from commercial hatcheries for this study. One thousand two hundred and sixty birds (1,260) from each species were weighed individually and randomly allocated to diets at 30 birds per cage and 6 replicate cages per diet. These birds were placed on the experimental diets from d 0 to d 5 when they were sampled. The remaining birds were fed a conventional maize-soybean meal-based starter diet appropriate for the respective species until day 16 when 336 birds were randomized to cages with 8 birds per cage. On day 21, the 336 birds were euthanized and ileal contents collected. All birds were euthanized using CO_2 asphyxiation.

Birds were raised in battery cages (Alternative design) till the end of the study in an environmentally controlled room with 24-h of light. Room temperature was 35 °C during the first week and was dropped by 5 degrees during the subsequent weeks. Birds had free access to feed and water. All animal care procedures were approved by the Purdue University Animal Care and Use Committee.

Sampling and ileal digesta processing

Content of the ileum (portion of the small intestine from Meckel's diverticulum to about 5 mm proximal to the ileo-cecal junction) was flushed with distilled water using 50 cL syringes (d 5) and wash bottles (d 21). Ileal digesta from birds within a cage were pooled and stored in a freezer (-20 °C) until they were processed. Samples were freeze-dried, ground using mortar and pestle, and were sent to the University of Missouri Experimental Station and Chemical Laboratory for complete amino acid profile and chromium analysis.

Chemical analyses

Prior to diet formulation, the N contents (%) of all the samples were determined on air dry basis. The percent crude protein (N x 6.25) was used to determine the level of inclusion of the feed ingredients in the diet. Dry matter content was determined on ground diets and ileal digesta by drying the samples at 100 °C for 24 h. Amino acids and chromium analyses were conducted at the University of Missouri Experiment Station and Chemical Laboratory. For amino acid analyses, samples were hydrolyzed in a 6 *N* HCl for 24 h at 110 °C under N atmosphere. For sulfur containing amino acids, methionine and cysteine, performic acid oxidation was carried out before acid hydrolysis. For tryptophan analysis, samples were hydrolyzed using barium hydroxide. The amino acids in the hydrolysate were then determined by HPLC after postcolumn derivatization (AOAC, 200; 982.30 E [a, b, c]. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Chromium was determined by the inductively coupled plasma atomic emission spectroscopy method (AOAC, 2000; 990.08) following nitric/perchloric acids wet ash digestion. Nitrogen in the various plant source feed ingredients was determined by the combustion method (AOAC, 2000; 990.03) (model FP2000, LECO Corp., St. Joseph, MI) using EDTA as a standard.

Calculations

Ileal EAA and TAA flow from both species was calculated as mg of amino acid and TAA flow per 1 kg of feed on DM intake basis using the following formula (Moughan et al., 1992):

Ileal (basal) amino acid flow (mg/kg DM intake) =

[amino acid in ileal digesta, mg/kg] x (diet chromium, mg/kg)/(ileal chromium, mg/kg)).

Standardized ileal amino acid digestibility, SIAAD, (%) = Apparent digestibility (%) + ((basal IEAA flow in g/kg DMI)/(AA content of the raw material in g/kg DM)) x100

Statistical analysis

Data were analyzed using the GLM Procedure of SAS (SAS Inst., Inc., Cary, NC). When the F-ratio is significant, treatment means were separated using Duncan multiple range test. The level of significance was set at $P < 0.05$.

RESULTS

The dietary composition and analyzed amino acid and N contents of the various diets are reported in Tables 1 and 2, respectively. Apparent ileal amino acid, TAA, and N digestibility in broiler chicks are reported in Table 3. The DM, TAA, and N digestibility were higher $(P < 0.05)$ on d 21 than on d 5. All the amino acids in the DDGS samples except tryptophan had higher $(P < 0.05)$ AD on d 21 (HD DDGS). There was no significant difference in lysine AD (75 vs. 78%, d 5 and d 21, respectively) in canola meal between d 5 and 21. All other amino acids showed higher $(P < 0.05)$ AD on d 21. For maize and SBM, AD for all the amino acids increased ($P < 0.05$) with age except for glutamic acid and serine in SBM where there was no age effect on AD. Overall, the lowest and highest N and TAA digestibility was seen in the low digestible DDGS and the SBM, respectively.

Standardized ileal amino acid, TAA, and N digestibility for both types of DDGS are shown in Table 4. For the HD DDGS, on d 5 method of standardization did not result in any significant difference in SIAAD except for isoleucine, glutamic acid, proline, and serine, which had higher $(P < 0.05)$ SIAAD value on d 21. Standardization of AD values for the HD DDGS using CDP on d 21 resulted in higher $(P < 0.05)$ SIAAD for TAA, N, and all amino acids except for phenylalanine and tyrosine. For the PD DDGS (Table 4), there was no significant effect of method of correction on SIAAD within the same age except for serine (on d 21) where CDP method of standardization resulted in increased $(P < 0.05)$ SIAAD.

Standardized ileal amino acid, TAA, and N digestibility for canola meal and maize are shown in Table 5. Standardization method resulted in differences $(P < 0.05)$ within age group for isoleucine, glutamic acid, and serine (d 5) and isoleucine, lysine, methionine, threonine, valine, alanine, aspartic acid, glutamic acid, proline, serine, TAA, and N (d 21) with higher $(P < 0.05)$ SIAAD values on d 21. For maize, standardization method did not result in any significant difference except for isoleucine, lysine, glutamic acid, proline, and serine (d 5). However, on d 21 standardization method resulted in higher $(P < 0.05)$ values for TAA, N, and all amino acids except arginine. Table 6 contains SIAAD values in broiler chicks for SBM. On d 5, there was no effect of standardization method on SIAAD values; however, by d 21, standardization using CDP resulted in significant difference in SIAAD values for isoleucine, methionine, valine, glutamic acid, proline, serine, TAA, and N.

The apparent amino acid digestibility of the various plant ingredients in turkey poults is reported in Table 7. Apparent digestibility for most of the amino acids improved ($P < 0.05$) with age except for the PD DDGS. Apparent digestibility of amino acids in the PD DDGS between d 5 and 21 are similar. For the HD DDGS and maize, all the amino acids, except tryptophan in the HD DDGS, showed significant improvement in AD with age. For canola meal and SBM, only six and 14 amino acids showed significant improvement with age. Overall, maize had the lowest total amino acid (TAA) digestibility on d 5 (48.8%) while the highest TAA digestibility on d 5 and 21 were seen in SBM. Only diets containing maize and SBM showed significant increase in dry matter digestibility with age (Table 7).

The SIAA and TAA digestibility values of the HD and PD DDGS in turkey poults are shown in Table 8. For the HD DDGS sample, SIAAD on d 5 and 21 were not significantly different for both methods of standardization. The difference in SIAAD between the two ages for the HD DDGS was about 2.9 (NFD) and 1.8 % (CDP) with age. However, for the low digestible DDGS, there was no difference in digestibility with age after standardization for all the amino acids. The difference in SIAAD between the two DDGS were 11.4 (NFD) and 11.2% (CDP)-d 5 and 19.7 (NFD) and 20.0% (CDP)-d 21.

Standardized ileal amino acid digestibility of canola meal and maize for turkey poults is shown in Table 9. Irrespective of the method of standardization, there was no difference in SIAAD on d 5 and 21. The SIAAD for maize as presented in Table 9 was not influence by the method of standardization on d 5. However, on d 21, the use of NFD for standardization resulted in higher ($P < 0.05$) SIAAD values for 11 of the amino acids. There was no effect of method of standardization on SIAAD of amino acids contained in SBM in poults (Table 10). These values were 5.8 (NFD) and 4.7% (CDP) higher on d 21.

DISCUSSION

A number of reasons have been adduced to the variations observed in amino acid composition of feed ingredients of plant origin. Factors leading to these variations include difference in variety, geographical location where the crop was cultivated, crop husbandry, seasonal variations, as well as year of harvest (Evers, et al., 1999; Jondreville, et al., 2001). Therefore, the digestibility values of these feed ingredients may vary depending on variations in any of the factors mentioned above.

The bulk of the data available on amino acid digestibility (total tract and ileal) of feed ingredients of plant origin are in broiler chickens (Huang et al., 2005; Kadim et al., 2002; Huang et al., 2006). However, it is important to note that the contribution of amino acids of endogenous origin to either the ileal or total tract digesta from feed ingredients may obscure the true amino acid digestibility values. Hence, there is the need to determine the SIAAD values of feed ingredients by correcting AD values for the basal endogenous amino acid contribution to the digesta. In this regard, there is a dearth of information on SIAAD values of feed ingredients in chicks and poults at young ages (d 5 and 21).

The effect of age on apparent ileal amino acid digestibility of the various plant feed ingredients evaluated in this study strongly support the fact that the digestibility of nearly all the amino acids increased with age (both species). This trend is similar to what Huang et al. (2005) reported for maize, canola meal, and SBM in broilers at 14, 28, and 42 d. However, d 21 amino acid digestibility values for maize and SBM in our study are similar to while that of canola meal are lower than what Huang et al. (2005) reported in 14 and 21-d-old broilers. The results from our broiler study (especially for methionine, lysine, threonine, and mean TAA) are similar to the amino acid digestibility for maize, canola meal, and SBM in 7 wk-old broiler chickens (Huang et al., 2006). The largest improvement from d 5 to 21 for methionine and lysine was in maize (chicks: Met, 31 and Lys, 35%; poults: Met, 29 and Lys, 41%). In the same vein, methionine improvement for the PD DDGS was about 32%. Threonine digestibility in chicks for in the PD DDGS also showed a huge improvement in apparent digestibility (~30%) with age. Unlike in chicks where all the amino acids in canola meal showed significant improvement in AD, with the exception of histidine, lysine, and tyrosine, only six amino acids in canola meal showed significant improvement with age in poults. With the exception of the HD DDGS where both species had similar TAA AD, chicks had a relatively higher apparent TAA digestibility at both ages that the poults. Also, for all the five plant source ingredients evaluated in this study, methionine and lysine digestibility on d 21 was relatively higher in chicks than in poults.

A number of reasons ranging from the level and activities of trypsin and chymotrypsin, to the rapid growth rate as well as the larger intestinal surface area per unit weight (Wakita, et al., 1970; Uni et al., 1995) have been given for the effects of age on AD of amino acids in feed ingredients. However, this study shows that the contribution of amino acids of endogenous origin play a significant role in the observed age effects on apparent digestibility. The endogenous amino acid contribution in this study was higher on d 5 than on d 21.

The presence of certain components of grain for instance the α-galactoside in SBM (Carre et al., 1995) have been reported to be poorly digested at younger ages, however, after standardization of the AD values, the difference disappeared in almost all the amino acids in both species and for both methods of standardization. Hence the difference in AD with age observed for SBM in this study between d 5 and 21 could be attributed mainly to the amino acids of endogenous origin.

Standardization in poults using either the NFD or the CDP methods did not result in any significant difference between the two methods within each age group for all the feed ingredients evaluated in this study except for maize where most of the amino acids were significantly higher on d 21 when standardization was by CDP method in chicks or by NFD method in poults. Likewise, standardization using CDP method resulted in higher digestibility values for PD DDGS and canola meal on d 21 (chicks). This becomes obvious because the apparent amino acids digestibility of the HD DDGS in poults were 11.4 (d 5, NFD) or 10.8% (d 5, CDP) and 7.7 (d 21 NFD) or 6.0% (d 21, CDP). Similar trend is seen for poults in the PD DDGS. The SIAAD for lysine and methionine (both species) for the HD DDGS in this study was similar to what Fastinger, et al (2006) reported for five different DDGS in adult roosters. Similarly, for the PD DDGS, the SIAAD for methionine and lysine were lower than what was reported by Fastinger, et al (2006). The relatively low AD and SIAAD for the PD DDGS in both species could be attributed to excessive heating during processing which ultimately may have resulted in the darker color of the DDGS (Parsons et al., 1992; Fastinger et al., 2006). The standardized TAA digestibility (mean of all the feed ingredients evaluated in this study) improved by about 7.4% (d 5) and 3.9% (d 21). The mean improvement (mean of NFD and CDP methods) in TAA in maize after standardization was about 11.2% (d 5) and 20% (d 21). The mean difference for threonine on d 5 and 21, respectively, were 24.5 and 12.4%. This shows that gain in digestibility with correction decreases from d 5 to d 21 in both species which again is an indication of a decrease in basal endogenous amino acid flow from d 5 to d 21. However, this improvement in SIAAD for TAA was higher in poults than in chicks (about doubled on d 21).

Another thing that can be inferred is that the diet specific endogenous amino acid flow between the two ages may not be significantly different. Unlike in the other feed ingredients whose mean standardized TAA digestibility value improved by about 11.5% (d 5) and 7.5% (d 21), the mean improvement (mean of NFD and CDP methods) over the AD values of TAA for maize after standardization was about 16% (d 5) and 6% (d 21) for chicks and 29% (d 5) and 20% (d 21) in poults. The mean difference for threonine in maize on d 5 and 21, respectively, were 25 and 12% (chicks) and 50 and 34% (for poults). This difference shows that standardization resulted in increased SIAAD and that this increase is more pronounced on d 5 in both species. This increase in digestibility was higher for poults than for chicks and is an indication of the fact that poults had higher basal endogenous amino acid flow than chicks, especially on d 5.

Standardized ileal amino acid digestibility values for maize in both species were very high compared to their respective apparent values. In some cases, these values were higher than 100% for both methods of correction. This is similar to what Kadim et al., (2002) reported for 5-wk-old broiler chicks. This clearly is not as a result for the method of standardization (NFD method) as suggested by Kadim et al. (2002) but may be as a result of the fact that since the dietary levels of the amino acids in corn is very low, such will make any calculations based on these value to be very sensitive, hence the high SIAAD values obtained in some cases.

The lack of differences between the two methods after correcting for the basal endogenous flow in most of the samples is an indication that both the NFD and CDP methods will give similar SIAAD value when used for standardization. In addition to this, comparison of SIAAD values (for each of the two methods) between d 5 and d 21 did not result in any significant difference (data not shown) except for amino acids in maize and four amino acids in the HD DDGS. In both cases, digestibility was higher on d 21. The lack of significant difference in SIAAD between the two ages shows that the bulk of the observed differences in AD values between the two ages were as a result of higher basal endogenous amino acid flow on d 5 compared to d 21. The observed difference in maize (both species) and some amino acids (histidine, cysteine, glutamic acid, and proline) in the HD DDGS after standardization (both methods of standardization in poults) could be attributed to the effects of diets specific endogenous amino acid flow which may have been produced at higher level on d 5. In chicks, however, the two DDGS and maize showed significant improvement in SIAAD with age. This means in addition to corn, the DDGS elicited a relatively higher diet specific endogenous amino acid flow on d 5 when compared to other feed ingredients in this study.

In summary, the AD of the feed ingredients evaluated in this study increased with age. Standardization using either the NFD or CDP resulted in similar SIAAD values within the same species and age group except for maize (d 21 poults) where NFD method resulted in higher SIAAD. In chicks however, standardization with CDP of the HD DDGS (d 21), canola meal (d 21) and maize (d 21) resulted in higher SIAAD values.

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Table 1. *Dietary composition of the experimental diets (on as is basis)*

	Diet[1]						
Ingredient g/kg	NFD	10% Casein	DDGS (HD)	DDGS (PD)	Canola	Maize	SBM
Maize starch	408	308	0	0	0	0	0
Dextrose	401	401	126	143	359	0	477
Amino acid source	0	100	767	750	537	923	419
Solkafloc	50	50	-	-	-	-	-
Soy oil	50	50	50	50	50	20	50
Vitamin-Mineral Premix[2]	5	5	5	5	5	5	5
Dicalcium phosphate	19	19	19	19	19	19	19
NaHCO3	20	20	.	.	.	.	.
KCl	12	12	.	.	.	.	.
MgO	2	2	.	.	.	.	.
Choline chloride	3	3	3	3	3	3	3
Limestone	13	13	13	13	10	13	10
NaCl (salt)	2	2	2	2	2	2	2
Chromic Ox Premix[3]	15	15	15	15	15	15	15
Total	1000	1000	1000	1000	1000	1000	1000

[1]NFD=Nitrogen-free diet; DDGS= Distiller dry grain soluble (highly or poorly digestible); SBM=Soybean meal

[2]Provided per kg of diet: Iron, 71.6 mg; Copper, 11.0 mg; Manganese, 178.7 mg; Zinc, 178.7 mg; Iodine, 3.0 mg; Selinium, 0.4 mg. Vitamin A (retinyl acetate), 18,904.3 IU; Vitamin D_3 (cholecalciferol), 9,480.0 IU; Vitamin E (DL-α-tocopheryl acetate), 63.0 IU; Vitamin K activity, 6.4 mg; Thiamine, 3.2 mg; Riboflavin, 9.4 mg; Pantothenic acid, 34.7 mg; Niacin, 126.0 mg; Pyridoxine, 4.7 mg; Folic acid, 1.6 mg; Biotin, 0.5 mg; Vitamin B_{12}, 35.4 mcg; choline, 956.9 mg.

[3]Chromic oxide (Cr_2O_3) premix added as index at a ratio 1:4 of chromic oxide:maize starch.

Table 2. *Analyzed amino acids and nitrogen composition of the experimental diets (on as is basis)*

	Diet[1]						
Item	NFD	CDP	DDGS (HD)	DDGS (PD)	Canola meal	Maize	Soybean meal
Essential amino acids, g/kg							
Arginine	0.0	3.2	9.0	6.7	11.0	3.7	16.5
Histidine	0.0	2.7	5.4	4.7	4.9	1.9	5.8
Isoleucine	0.1	4.9	7.7	7.4	7.4	2.6	10.2
Leucine	0.2	9.0	24.2	23.5	13.6	8.3	17.4
Lysine	0.1	7.4	6.5	4.0	11.2	2.4	14.1
Methionine	0.0	2.1	3.5	3.2	3.1	1.2	2.7
Phenylalanine	0.1	4.8	10.0	9.6	7.6	3.5	11.3
Threonine	0.1	3.9	7.7	6.9	7.8	2.7	8.6
Tryptophan	< 0.4	1.1	1.7	1.0	2.3	0.6	3.5
Valine	0.1	6.1	10.0	9.4	9.5	3.3	10.7
Nonessential amino acids, g/kg							
Alanine	0.1	2.9	14.7	14.1	8.4	5.1	9.7
Aspartic acid	0.1	6.6	12.9	11.8	13.3	4.7	25.3
Cysteine	0.2	0.6	3.8	3.6	4.3	1.8	3.4
Glutamic acid	0.3	20.5	31.7	31.7	31.9	12.7	41.1
Glycine	0.1	1.8	8.0	7.3	9.2	2.9	9.5
Hydroxyproline	0.0	0.0	2.9	1.7	1.6	0.1	0.5
Proline	0.1	9.4	15.9	15.2	11.0	5.7	11.2
Serine	0.1	4.7	9.6	8.5	7.5	3.3	10.8
Tyrosine	0.1	4.4	7.7	7.1	5.3	2.7	7.7
Total amino acids	3.0	97.2	193.9	178.1	171.9	70.4	221.0
Nitrogen	0.0	11.9	32.3	30.2	29.1	11.0	35.2

NFD= Nitrogen-free diet; CDP= Completely digestible protein; DDGS= Distillers dried grain soluble (HD=Highly digestible; PD= Poorly digestible).

Table 3. *Apparent ileal amino acid, total amino acid, and nitrogen digestibility in broiler chicks fed diets containing amino acids of plant origin (on DM basis, days 5 and 21)*[1]

	Apparent digestibility, %														
Age, days	5	21		5	21		5	21		5	21		5	21	
Item	DDGS[2] (HD)	DDGS (HD)	SD	DDGS (PD	DDGS (PD	SD	Canola	Canola	SD	Maize	Maize	SD	SBM	SBM	SD
N[3]	6	6		6	6		6	6		6	6		6	6	
Dry matter	56.33^{b}	61.50^{a}	1.756	51.37^{b}	63.57^{a}	3.684	64.17^{b}	68.67^{a}	1.794	75.33^{b}	84.33^{a}	2.017	72.67^{b}	80.00^{a}	3.246
Essential amino acid															
Arginine	72.50^{b}	81.00^{a}	1.830	45.92^{b}	73.02^{a}	6.370	82.00^{b}	84.67^{a}	1.528	69.00^{b}	89.00^{a}	3.130	86.83^{b}	90.83^{a}	2.787
Histidine	67.67^{b}	76.17^{a}	1.489	42.47^{b}	65.67^{a}	7.782	79.67	81.50	1.812	65.50^{b}	88.17^{a}	3.039	83.67^{b}	88.17^{a}	3.133
Isoleucine	67.83^{b}	76.33^{a}	1.848	46.68^{b}	71.60^{a}	8.357	73.83^{b}	76.67^{a}	1.794	65.83^{b}	87.17^{a}	3.629	81.33^{b}	86.83^{a}	3.524
Leucine	78.00^{b}	86.33^{a}	1.065	58.10^{b}	79.75^{a}	6.052	76.83^{b}	78.83^{a}	1.472	76.33^{b}	91.83^{a}	2.004	81.50^{b}	86.00^{a}	3.279
Lysine	51.33^{b}	63.33^{a}	3.751	26.00^{b}	48.67^{a}	9.680	75.17	78.05	2.262	50.33^{b}	85.00^{a}	6.923	83.50^{b}	88.67^{a}	3.418
Methionine	65.67^{b}	84.00^{a}	2.033	45.63^{b}	78.53^{a}	5.491	76.50^{b}	84.00^{a}	1.718	59.83^{b}	91.17^{a}	4.401	79.50^{b}	90.00^{a}	3.654
Phenylalanine	73.67^{b}	82.00^{a}	1.317	53.87^{b}	76.62^{a}	6.130	76.33^{b}	78.67^{a}	1.506	70.00^{b}	89.33^{a}	3.055	81.67^{b}	86.67^{a}	3.296
Threonine	56.50^{b}	69.33^{a}	2.299	30.70^{b}	60.37^{a}	6.591	65.67^{b}	71.17^{a}	2.453	47.33^{b}	76.00^{a}	5.910	73.50^{b}	81.33^{a}	4.205
Tryptophan	76.67	78.50	1.638	51.83^{b}	65.97^{a}	5.923	91.33^{b}	87.50^{a}	1.372	70.83^{b}	85.33^{a}	3.580	89.67^{b}	92.17^{a}	1.618
Valine	65.83^{b}	75.50^{a}	1.494	42.58^{b}	69.05^{a}	8.142	72.67^{b}	76.17^{a}	1.794	59.33^{b}	84.17^{a}	4.384	79.50^{b}	85.17^{a}	3.582
Nonessential amino acid															
Alanine	74.83^{b}	83.67^{a}	1.190	55.33^{b}	76.85^{a}	5.525	75.17^{b}	78.50^{a}	1.958	74.00^{b}	90.50^{a}	2.313	79.67^{b}	85.50^{a}	3.806
Aspartic acid	59.00^{b}	69.67^{a}	2.309	33.32^{b}	59.27^{a}	7.517	72.00^{b}	75.00^{a}	2.098	59.50^{b}	83.67^{a}	4.481	79.67^{b}	84.83^{a}	3.926
Cysteine	65.33^{b}	74.17^{a}	1.678	44.22^{b}	64.55^{a}	6.515	72.00^{b}	76.00^{a}	1.949	67.67^{b}	85.17^{a}	2.935	69.83^{b}	79.33^{a}	5.641
Glutamic acid	75.17^{b}	84.33^{a}	1.190	54.93^{b}	77.03^{a}	5.585	83.50^{b}	86.17^{a}	1.426	77.67^{b}	91.67^{a}	2.066	85.00	88.50	2.924
Glycine	60.50^{b}	69.83^{a}	1.742	37.38^{b}	60.73^{a}	6.827	71.67^{b}	77.00^{a}	2.129	57.67^{b}	80.83^{a}	3.926	76.33^{b}	83.33^{a}	4.227
Proline	69.33^{b}	82.33^{a}	1.033	51.00^{b}	73.80^{a}	5.751	68.83^{b}	76.00^{a}	2.585	67.50^{b}	88.50^{a}	2.811	77.17^{b}	86.00^{a}	4.721
Serine	67.00^{b}	79.67^{a}	1.713	45.35^{b}	71.33^{a}	5.127	70.83^{b}	73.83^{a}	2.041	62.33^{b}	82.67^{a}	4.590	81.00	84.00	3.633
Tyrosine	75.67^{b}	83.17^{a}	1.420	56.83^{b}	77.68^{a}	5.489	75.00	76.50	1.775	70.83^{b}	88.33^{a}	2.866	82.66^{b}	86.83^{a}	3.101
Total amino acid[4]	69.00^{b}	79.00^{a}	1.483	46.18^{b}	71.12^{a}	6.234	75.17^{b}	78.00^{a}	1.812	66.50^{b}	89.17^{a}	3.498	81.00^{b}	86.00^{a}	3.578
Nitrogen	64.67^{b}	73.67^{a}	1.966	40.32^{b}	63.67^{a}	5.749	67.50^{b}	72.00^{a}	2.636	54.17^{b}	80.00^{a}	3.447	75.83^{b}	83.67^{a}	3.717

[a-b] Means within the same row and within the same treatment with different superscripts are significantly different ($P < 0.05$).
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Distillers dry grain soluble (HD=highly digestible; PD=poorly digestible).
[3]Number of replicates.
[4]Means of 18 amino acids.

Table 4. *Standardized ileal amino acid, total amino acid, and nitrogen digestibility in broiler chicks fed diets containing amino acids of plant origin*[1]

	Distillers dried grain soluble (highly digestible)						Distillers dried grain soluble (poorly digestible)					
Age, days	5			21			5			21		
Method	NFD[2]	CDP[3]	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6		6	6		6	6	
Essential amino acid												
Arginine	77.50	78.00	2.398	82.33^{b}	83.83^{a}	1.008	53.00	53.50	7.652	74.83	76.50	4.861
Histidine	71.67	72.67	1.633	77.17^{b}	79.00^{a}	1.297	47.00	48.33	8.771	66.83	69.00	6.788
Isoleucine	72.83^{b}	76.00^{a}	2.210	77.83^{b}	82.67^{a}	0.904	52.00	55.50	10.495	73.17	78.33	5.331
Leucine	80.83	81.17	1.472	86.83^{b}	87.83^{a}	0.753	64.33	61.67	7.776	80.50	81.67	3.533
Lysine	59.62	63.20	4.758	65.33^{b}	70.33^{a}	2.251	31.33	37.33	14.364	52.00	59.67	10.504
Methionine	72.17	73.67	2.611	85.33^{b}	88.50^{a}	1.218	53.17	54.83	6.882	79.83	83.50	3.773
Phenylalanine	77.83	78.33	1.737	83.33	84.17	0.903	58.33	59.00	7.677	77.67	78.67	3.933
Threonine	63.67	65.50	2.772	72.17^{b}	75.00^{a}	1.372	39.17	41.17	7.026	63.50	66.83	6.069
Tryptophan	81.50	82.00	1.718	NA	82.33	1.506	60.17	61.00	7.161	NA	72.67	4.676
Valine	71.50	73.50	1.871	77.33b	80.33^{a}	1.211	48.83	50.83	10.048	70.83	74.17	5.529
Nonessential amino acid												
Alanine	78.50	78.67	1.443	84.50^{b}	85.83^{a}	0.658	59.17	59.50	7.088	77.67	79.17	3.635
Aspartic acid	65.33	67.00	2.817	71.67^{b}	74.67^{a}	1.633	40.33	42.17	8.403	61.17	64.50	6.406
Cysteine	71.03	71.14	1.941	76.17^{b}	77.67^{a}	0.904	50.40	50.52	7.369	66.67	68.50	5.872
Glutamic acid	78.33^{b}	82.00^{a}	1.653	85.33^{b}	89.50^{a}	0.695	58.67	62.00	6.865	78.00	82.00	4.937
Glycine	66.37	66.46	2.168	71.50^{b}	73.17^{a}	1.111	43.90	43.98	7.668	62.83	64.50	5.953
Proline	72.17^{b}	74.17^{a}	1.472	83.33^{b}	85.00^{a}	0.730	53.83	55.83	7.333	74.67	76.67	3.724
Serine	72.33^{b}	75.50^{a}	2.384	81.17^{b}	86.67^{a}	0.785	51.50	55.50	5.718	73.00^{b}	79.50^{a}	4.577
Tyrosine	79.67	80.67	1.633	84.67	85.67	0.816	61.33	62.50	6.862	78.83	80.50	3.799
Total amino acid[5]	74.00	75.67	1.770	80.67^{b}	83.00^{a}	0.856	51.67	53.50	7.529	72.83	75.83	4.535
Nitrogen	70.67	72.17	2.611	75.83^{b}	78.67^{a}	1.103	47.50	48.67	6.394	66.33	68.83	5.081

$^{a\text{-}b}$ Means within the same row and within the same treatment and age with different superscripts are significantly different ($P < 0.05$).
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids.

Table 5. *Standardized ileal amino acid, total amino acid, and nitrogen digestibility in broiler chicks fed diets containing amino acids of plant origin*[1]

	Canola meal						Maize					
Age, days	5			21			5			21		
Method	NFD[2]	CDP[3]	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6		6	6		6	6	
Essential amino acid												
Arginine	86.17	86.83	1.835	85.67	86.67	1.366	82.00	83.00	3.521	92.17	95.50	2.652
Histidine	84.33	85.67	1.915	82.50	84.50	1.643	76.50	80.00	3.599	88.33^b	94.00^a	3.596
Isoleucine	79.33^b	82.83^a	2.195	78.00^b	83.50^a	1.718	81.17^b	91.00^a	4.322	91.33^b	106.33^a	2.338
Leucine	82.50	83.50	1.871	80.17	82.00	1.443	85.17	87.17	1.835	93.83^b	96.83^a	2.041
Lysine	80.00	82.33	2.633	79.17^b	82.00^a	1.919	73.17^b	83.17^a	7.731	86.33^b	99.00^a	4.747
Methionine	84.17	86.17	2.137	85.33^b	89.17^a	1.555	78.83	83.50	5.851	95.17^b	104.17^a	2.401
Phenylalanine	82.33	83.17	1.954	80.17	81.33	1.489	82.00	84.00	3.633	92.33^b	95.33^a	2.338
Threonine	73.50	75.17	2.799	73.83^b	76.83^a	1.329	69.50	74.17	7.002	84.00^b	92.83^a	4.368
Tryptophan	95.00	95.33	1.065	NA	90.33	1.633	84.83	86.00	2.624	NA	96.33	4.320
Valine	79.17	81.17	2.483	77.50^b	81.17^a	1.560	76.83	82.50	5.238	89.00^b	98.83^a	3.329
Nonessential amino acid												
Alanine	81.33	82.17	2.240	80.00^b	82.33^a	1.653	84.33	85.50	2.913	93.00^b	97.00^a	2.280
Aspartic acid	78.33	80.00	2.556	76.83^b	80.00^a	1.576	77.50	81.83	5.480	88.17^b	96.83^a	3.281
Cysteine	77.17	77.27	2.449	78.00	79.33	1.317	80.05	80.29	3.430	89.50^b	92.83a	2.576
Glutamic acid	87.33^b	90.50^a	1.638	87.17^b	91.50^a	1.111	86.67^b	95.17^a	2.240	94.17^b	104.67^a	2.149
Glycine	76.83	77.00	2.585	78.83	80.17	1.663	73.67	74.17	4.291	85.67^b	90.33^a	3.416
Proline	73.33	76.00	3.276	77.33^b	80.00^a	1.592	75.50^b	80.67^a	3.533	91.00^b	96.00^a	2.280
Serine	78.17^b	82.67^a	2.611	75.83^b	83.33^a	1.794	78.33^b	88.17^a	5.311	87.33^b	103.67^a	4.033
Tyrosine	81.00	82.67	1.983	78.33	79.83	1.348	82.67	85.83	3.379	91.33^b	94.83^a	2.533
Total amino acid[5]	81.00	82.83	2.210	79.83^b	82.83^a	1.472	80.50	85.00	3.814	91.00^b	98.33^a	2.817
Nitrogen	75.00	76.33	3.396	74.83^b	77.83^a	1.835	73.83	77.17	4.095	87.50^b	94.83^a	2.726

[a-b] Means within the same row and within the same treatment and age with different superscripts are significantly different ($P < 0.05$).
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids.

Table 6. *Standardized ileal amino acid, total amino acid, and nitrogen digestibility in broiler chicks fed diets containing amino acids of plant origin*[1]

	Soybean meal					
Age, days		5			21	
Method	NFD[2]	CDP[3]	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6	
Essential amino acid						
Arginine	89.67	89.83	3.849	91.50	92.17	1.494
Histidine	87.50	88.50	3.886	89.17	90.83	1.472
Isoleucine	85.17	87.67	4.406	87.83^{b}	91.67^{a}	1.902
Leucine	85.67	86.67	4.367	87.00	88.67	1.983
Lysine	87.33	89.00	4.187	89.50	91.67	1.866
Methionine	88.17	90.33	4.496	91.50^{b}	95.83^{a}	2.331
Phenylalanine	85.67	86.17	4.361	87.50	88.50	1.761
Threonine	80.50	81.83	5.276	83.67	86.33	2.422
Tryptophan	92.50	92.67	2.210	NA	94.17	0.753
Valine	84.83	86.83	4.665	87.00^{b}	90.00^{a}	2.000
Nonessential amino acid						
Alanine	85.00	86.00	4.648	86.83	88.83	2.137
Aspartic acid	82.67	83.67	5.203	85.83	87.50	2.008
Cysteine	76.12	76.29	7.430	81.83	83.33	2.533
Glutamic acid	88.00	90.83	3.909	89.50^{b}	92.50^{a}	1.517
Glycine	81.39	81.46	5.574	85.00	86.33	2.082
Proline	81.33	84.00	6.460	87.50^{b}	90.00^{a}	1.658
Serine	85.83	89.00	4.437	85.67^{b}	90.67^{a}	2.160
Tyrosine	86.83	87.83	4.262	88.00	89.17	1.866
Total amino acid[5]	85.33	87.00	4.683	87.33^{b}	90.00^{a}	1.826
Nitrogen	82.17	83.33	5.293	86.33^{b}	88.50^{a}	1.443

[a-b] Means within the same row and within the same treatment and age with different superscripts are significantly different *($P < 0.05$).*
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids.

Table 7. *Apparent ileal amino acid and total amino acid digestibility in poults fed diets containing amino acids of plant origin (on DM basis, d 5 and 21)*[1]

	Apparent digestibility, %														
Age, days	5	21		5	21		5	21		5	21		5	21	
	DDGS[2] (HD)	DDGS (HD)	SD	DDGS (PD)	DDGS (PD)	SD	Canola	Canola	SD	Maize	Maize	SD	SBM	SBM	SD
N[3]	5	6		5	3		6	6		5	4		6	6	
Dry matter	49.40	55.00	4.361	50.20	48.00	5.785	61.33	63.83	3.552	62.60^{b}	77.75^{a}	3.294	68.50^{b}	77.33^{a}	3.175
Essential amino acid															
Arginine	70.60^{b}	75.50^{a}	3.175	55.40	56.50	5.324	80.67	83.17	2.831	51.20^{b}	74.75^{a}	4.302	82.50^{b}	88.67^{a}	4.481
Histidine	67.00^{b}	74.17^{a}	3.142	51.20	55.33	5.937	74.00^{b}	78.00^{a}	2.933	48.00^{b}	71.50^{a}	4.551	74.67	82.33	6.346
Isoleucine	67.60^{b}	72.50^{a}	3.175	52.80	49.00	7.151	67.50	71.00	3.841	36.80^{b}	69.75^{a}	6.563	70.67^{b}	81.00^{a}	7.559
Leucine	77.80^{b}	82.50^{a}	1.894	65.60	67.67	3.263	70.50	74.33	3.476	66.00^{b}	83.00^{a}	3.207	69.83^{b}	79.33^{a}	7.129
Lysine	48.20	55.33	6.020	23.8	19.0	11.034	71.33	73.00	4.305	14.00^{b}	55.25^{a}	7.697	75.50	84.17	6.873
Methionine	69.00^{b}	76.83^{a}	3.664	65.80	62.67	7.365	76.17^{b}	82.17^{a}	3.629	50.20^{b}	79.00^{a}	5.865	63.50^{b}	80.67^{a}	10.976
Phenylalanine	72.80^{b}	77.50^{a}	1.894	61.20	58.00	6.841	70.00^{b}	75.00^{a}	3.661	53.20^{b}	76.00^{a}	4.290	71.67^{b}	81.67^{a}	6.608
Threonine	53.80^{b}	61.83^{a}	2.937	40.60	37.67	6.707	58.50^{b}	65.67^{a}	4.700	13.40^{b}	49.00^{a}	5.846	59.17^{b}	73.83^{a}	9.207
Tryptophan	65.60	71.17	4.989	61.40	53.33	9.625	87.00	88.33	2.745	48.80^{b}	71.50^{a}	5.877	86.33	89.17	2.866
Valine	61.60^{b}	69.50^{a}	3.278	46.60	47.67	5.064	63.00	68.50	4.399	29.40^{b}	64.25^{a}	5.554	62.83^{b}	76.83^{a}	8.807
Nonessential amino acid															
Alanine	72.60^{b}	77.67^{a}	1.901	61.80	64.67	3.040	70.83	74.50	3.610	62.00^{b}	80.25^{a}	2.745	66.67^{b}	77.17^{a}	8.125
Aspartic acid	52.40^{b}	59.17^{a}	3.652	42.00	40.50	5.301	67.50	71.83	3.954	34.60^{b}	66.50^{a}	6.097	71.83^{b}	82.00^{a}	6.847
Cysteine	48.80^{b}	63.33^{a}	3.889	40.20	42.50	4.632	62.67^{b}	69.50^{a}	3.299	34.40^{b}	67.25^{a}	5.450	46.17^{b}	71.00^{a}	12.739
Glutamic acid	68.00^{b}	75.33^{a}	2.893	57.40	56.67	5.194	82.33	84.17	2.370	66.60^{b}	83.50^{a}	2.833	79.67	86.33	5.183
Glycine	53.80^{b}	60.67^{a}	3.657	40.40	37.33	4.759	70.17	73.83	2.893	31.00^{b}	59.00^{a}	4.243	64.17^{b}	76.67^{a}	7.708
Proline	71.40^{b}	78.83^{a}	1.945	63.20	66.33	3.201	68.50	71.50	2.510	67.20^{b}	80.75^{a}	2.377	70.83^{b}	80.50^{a}	6.590
Serine	63.40^{b}	72.00^{a}	3.933	51.60	49.33	7.658	57.83^{b}	67.83^{a}	5.307	37.60^{b}	67.25^{a}	4.275	67.50^{b}	79.00^{a}	8.219
Tyrosine	73.80^{b}	78.83^{a}	1.815	64.80	64.00	4.099	67.33	72.50	4.060	52.40^{b}	75.00^{a}	3.649	72.67^{b}	82.50^{a}	6.905
Total amino acid[4]	65.40^{b}	72.00^{a}	2.733	53.00	51.67	5.547	69.67^{b}	74.00^{a}	3.152	48.80^{b}	72.00^{a}	4.323	71.33^{b}	81.17^{a}	6.650

[a-b] Means within the same row and within the same treatment with different superscripts are significantly different ($P < 0.05$).

[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.

[2]Distillers dry grain soluble (HD=highly digestible; PD=poorly digestible).

[3]Number of replicates.

[4]Means of 18 amino acids.

Table 8. *Standardized ileal amino acid and total amino acid digestibility in poults fed diets containing amino acids of plant origin*[1]

	Distillers dried grain soluble (highly digestible)						Distillers dried grain soluble (poorly digestible)					
Age, days	5			21			5			21		
Method	NFD[2]	CDP[3]	SD	NFD	CDP	SD	NFD	CDP	SD	NFD	CDP	SD
N[4]	5	5		6	6		5	5		3	3	
Essential amino acid												
Arginine	81.20	80.20	1.304	82.17	80.00	4.109	70.40	69.20	2.784	66.00	62.50	10.259
Histidine	75.80	75.40	3.354	79.67	78.67	3.011	62.20	62.00	5.753	62.33	61.00	6.218
Isoleucine	78.80	78.60	2.550	79.00	79.00	3.899	65.60	65.60	4.037	57.00	57.00	10.817
Leucine	84.40	83.80	1.323	86.50	85.50	2.258	73.00	72.40	2.855	72.00	70.67	3.697
Lysine	65.70	65.62	2.794	66.50	64.50	7.969	54.00	54.00	5.1478	35.50	38.50	21.920
Methionine	81.40	80.80	3.202	82.33	81.50	3.779	77.80	76.80	2.950	68.33	67.33	12.583
Phenylalanine	82.00	81.00	1.581	81.00	83.17	2.661	72.00	70.80	2.665	64.67	62.00	11.098
Threonine	69.80	68.80	2.387	72.50	69.83	3.230	60.00	58.60	3.413	50.67	47.67	10.693
Tryptophan	80.80	75.80	4.658	82.67	78.17	5.022	83.60[a]	76.40[b]	2.702	69.67	63.00	16.238
Valine	74.60	74.00	2.480	77.33	75.83	3.524	61.40	60.60	4.904	56.67	55.67	5.033
Nonessential amino acid												
Alanine	80.40	79.80	1.323	82.67	81.67	2.338	69.80	69.60	3.082	69.67	68.67	3.215
Aspartic acid	68.40	67.60	2.191	69.00	66.83	4.570	59.00	58.20	3.619	50.50	48.50	9.192
Cysteine	66.20	63.60	3.571	73.67	70.00	3.941	58.20	55.20	3.421	53.50	49.50	7.778
Glutamic acid	77.80	78.60	1.225	81.67	82.33	3.502	66.60	67.20	3.742	62.00	63.00	7.550
Glycine	66.00	65.00	2.345	69.33	66.67	3.724	54.60	53.40	3.612	47.33	44.33	6.658
Proline	78.00	78.00	1.871	83.67	82.67	2.066	70.20	70.20	3.271	71.00	70.00	2.646
Serine	78.00	78.00	3.937	80.50	81.00	3.762	69.60	69.60	4.450	59.33	60.00	11.902
Tyrosine	82.60	81.60	1.342	83.83	81.83	2.229	74.60	73.80	2.000	70.00	67.67	6.795
Total amino acid[5]	76.80	76.20	1.924	79.67	78.00	3.307	65.40	65.00	3.592	60.00	58.00	8.185

[a-b] Means within the same row and within the same treatment and age with different superscripts are significantly different ($P < 0.05$).
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids.

Table 9. *Standardized ileal amino acid and total amino acid digestibility in poults fed diets containing amino acids of plant origin*[1]

	Canola meal						Maize					
Age, days	5			21			5			21		
Method	NFD[2]	CDP[3]	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6		6	6		4	4	
Essential amino acid												
Arginine	90.33	89.33	2.658	89.17	87.17	2.927	79.00	76.00	10.714	94.75[a]	87.75[b]	2.630
Histidine	85.00	84.67	1.826	84.50	83.00	3.681	75.67	74.67	8.756	90.75	86.50	3.553
Isoleucine	80.83	80.67	3.849	79.00	79.00	3.795	74.33	73.50	15.234	95.50	95.50	3.109
Leucine	83.17	82.17	3.920	82.17	79.83	3.430	84.33	82.33	8.311	95.75[a]	91.75[b]	1.258
Lysine	82.33	82.33	3.445	80.17	79.00	5.088	63.00	63.00	21.157	91.25[a]	85.50[b]	2.508
Methionine	88.83	88.33	4.197	88.00	87.17	3.299	77.17	75.17	16.167	93.75	91.75	2.986
Phenylalanine	83.83	82.33	3.926	83.33	80.00	3.327	79.83	76.00	11.674	94.25[a]	87.25[b]	2.500
Threonine	76.00	74.83	4.988	77.33	74.33	3.670	66.00	61.83	15.142	88.00[a]	78.00[b]	4.243
Tryptophan	97.17	93.83	3.157	96.00	93.00	2.449	87.83	73.67	14.114	104.00[a]	91.00[b]	3.916
Valine	78.00	77.17	5.068	77.67	76.33	3.474	70.33	68.17	14.269	92.25	88.00	2.475
Nonessential amino acid												
Alanine	84.00	83.67	3.651	83.00	81.00	3.578	81.33	80.67	7.444	95.00[a]	91.50[b]	1.354
Aspartic acid	82.83	82.33	4.197	81.17	79.17	3.869	74.17	72.17	14.552	94.00[a]	88.00[b]	2.582
Cysteine	77.83	75.17	3.545	78.33	75.33	3.077	73.50	66.50	9.711	91.50[a]	83.50[b]	2.380
Glutamic acid	90.33	90.83	2.240	89.33	90.00	2.436	84.67	86.50	7.105	96.50	98.50	1.291
Glycine	81.17	80.33	2.686	81.33	79.33	2.943	67.33	64.17	8.603	86.25[a]	78.75[b]	3.304
Proline	77.50	77.67	2.384	78.17	76.67	2.796	83.00	83.67	4.305	93.25	91.00	1.860
Serine	78.17	78.50	5.257	79.33	79.83	5.274	77.50	77.83	13.922	93.25	94.75	2.432
Tyrosine	81.33	79.83	4.496	81.00	78.00	3.578	78.83	76.33	11.359	93.50[a]	87.00[b]	2.041
Total amino acid[5]	82.50	82.00	3.457	82.83	80.83	3.061	78.33	76.83	9.890	94.50[a]	89.50[b]	2.887

[a-b] Means within the same row and within the same treatment and age with different superscripts are significantly different ($P < 0.05$).
[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids.

Table 10. *Standardized ileal amino acid and total amino acid digestibility s in poults fed diets containing amino acids of plant origin*[1]

	Soybean meal					
Age, days		5			21	
Method	NFD[2]	CDP[3]	SD	NFD	CDP	SD
N[4]	6	6		6	6	
Essential amino acid						
Arginine	90.33	89.50	5.557	93.33	92.00	3.276
Histidine	85.83	85.67	7.511	89.33	88.00	4.872
Isoleucine	82.33	82.33	9.266	87.83	87.83	5.492
Leucine	81.67	80.50	8.700	84.17	86.50	4.778
Lysine	86.00	85.83	7.777	90.67	89.50	5.430
Methionine	84.67	83.50	13.671	90.67	88.67	7.528
Phenylalanine	82.83	81.33	8.451	88.17	85.83	4.622
Threonine	78.17	76.67	11.714	86.67	83.67	5.955
Tryptophan	95.50	92.50	2.881	96.00	93.33	2.633
Valine	79.17	78.50	11.200	86.83	85.17	5.964
Nonessential amino acid						
Alanine	80.67	79.67	9.913	86.17	84.17	5.811
Aspartic acid	81.67	81.17	8.113	88.00	86.83	4.805
Cysteine	69.83	66.00	16.562	85.00	80.17	7.327
Glutamic acid	87.00	87.67	5.994	91.17	91.67	4.076
Glycine	77.00	76.00	9.402	85.67	83.00	5.360
Proline	81.83	82.33	8.199	88.33	86.67	4.546
Serine	83.67	83.67	9.993	88.50	88.83	6.560
Tyrosine	83.67	82.83	8.787	89.50	87.33	4.276
Total amino acid[5]	83.33	82.67	8.214	89.17	87.33	4.671

[1]Values are means of 6 replicate cages of 30 birds per cage at day 5 and 8 birds per cage at day 21.
[2]Nitrogen-free diet.
[3]Completely digestible protein (10% casein/kg diet).
[4]Number of replicates.
[5]Means of 18 amino acids .

Section: Metabolism and nutrition

Standardized Ileal Amino Acid Digestibility of Meat and Bone Meal in Broiler Chicks and Turkey Poults using a Nitrogen-free or Casein diet[1]

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Abbreviation Key: AID =Apparent ilea digestibility, CDP = Completely digestible protein, MBM = Meat and bone meal, NFD = Nitrogen-free diet, SIAAD = Standardized ileal amino acid digestibility, TAA = Total amino acid

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Running Title: STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY

Section: Metabolism and nutrition

Standardized Ileal Amino Acid Digestibility of Meat and Bone Meal in Broiler Chicks and Turkey Poults using a Nitrogen-free or Casein diet

ABSTRACT The aim of this study was to determine the effect of bird age, standardization method, and sources of meat and bone meal (MBM) on standardized ileal amino acid digestibility (SIAAD). The SIAAD were obtained by correcting apparent ileal digestibility values for basal ileal endogenous amino acid (IEAA) flow obtained from broiler chicks and turkey poults fed a N-free diet (NFD) or a completely digestible protein (CDP) diet containing 100 g casein/kg diet. Each of the 4 diets was formulated to contain 20 % crude protein (CP) using MBM as the only source of CP. Diets were fed for 5 d prior to sampling with 6 replicate cages of 30 or 8 birds/cage at 5 and 21 d, respectively. With the exception of MBM1 (all beef), chick apparent ileal digestibility (AID) on d 21 was higher *($P < 0.05$)* than on d 5. However, AID in poults was not significantly different between the two ages with the exception of histidine whose AID was higher *($P < 0.05$)* at d 21. There was no significant difference in SIAAD between the two methods within d 5 or d 21 for both species. Comparing both methods of standardization, the SIAAD for methionine (MBM1, all beef) was 75.8%-NFD or 82.7%-CDP (d 5) and 69.5%-NFD or 74.5%-CDP (d 21). The corresponding values for turkey poults were 63.8%-NFD or 69.8%-CDP (d 5) and 70.2%-NFD or 78.0%-CDP (d 21). The SIAAD for methionine (MBM2, all pork) was 54.5%-NFD or 59.3%-CDP (d 5) and 76.3%-NFD or 79.8%-CDP (d 21). For the poults, the corresponding values were 79.0%-NFD or 84.0%-CDP (d 5) and 84.8%-NFD or 91.8%-CDP (d 21). For MBM3 (mixed species), the SIAAD for methionine was 50.2%-NFD or 55.2%-CDP (d 5) and 70.7%-NFD or 74.2%-CDP (d 21). The corresponding values for turkey poults were 71.4%-NFD or 76.4%-CDP (d 5) and 72.8%-NFD or 79.3%-CDP (d 21). The SIAAD for methionine from MBM4 (mixed species) was 66.4%-NFD or 70.4%-CDP (d 5) and 75.3%-NFD or 78.3%-CDP (d 21). The corresponding values for turkey poults were 78.4%-NFD or 82.4%-CDP (d 5) and 86.4%-NFD or 91.4%-CDP (d 21). The of variation in SIAAD in chicks between the two methods of standardization was higher on d 5 (8.1%) relative to d 21 (5.0%) for all the treatments. In poults a higher variation was seen on d 21 (8.2%) relative to d 5 (6.7%). Results from this study showed that SIAAD using either NFD or CDP diets resulted in similar digestibility values. The margin of variation between the two methods, however, decreased with age.

(Word count 417 as against 325 words)

(*Key words*: Casein, chick, ileal endogenous amino acid, meat and bone meal, poult, standardized ileal digestibility)

INTRODUCTION

For optimum poultry production it is important to formulate diets to meet the birds' requirements while at the same time reducing the amount of nutrients that is excreted into the environment. For these objectives to be achieved, it is important to base diet formulation on standardized ileal digestibility rather than on apparent ileal digestibility. To achieve this goal, accurate information on amino acids digestibility in feed ingredients is required. Despite the fact that there is huge information in the literature on digestibility of nutrients in feedstuffs for poultry, most of these do not take into account endogenous amino acid (EAA) contributions to ileal digesta or excreta. Basing diet formulations on apparent ilea digestibility (AID), it is obvious that digestibility of amino acids in feedstuffs is underestimated hence this may result in increased level of nutrient excretion.

Ileal EAA and total amino acid (TAA) contribution to the digesta originate from various digestive secretions which include the saliva, bile, pancreatic secretions, gastric secretions, and intestinal secretions. Other sources of endogenous amino acids are mucoproteins and desquamated epithelial cell from the intestinal lining as well as nitrogen from non-dietary origin that arise from exocrine pancreatic secretions (Greene, et al., 1963; Nyachoti, 1997; Ravindran and Bryden, 1999). By correcting for basal IEAA and TAA secretions using N-free diet (NFD) and completely digestible protein (CDP) methods, the standardized ileal amino acid digestibility (SIAAD) obtained will be close to the true digestibility value leaving only the diet specific EAA loss uncorrected for. A number of techniques have been used to estimate IEAA flow. Each of these techniques has its strengths and weaknesses. According to Huang et al. (2005), apparent ileal digestibility of crude protein and amino acids is affected by age of the bird, however, these effects varied among amino acids and ingredients.

A number of factors have been reported to contribute to the apparent ileal amino acid digestibility. These factors range from the level of inclusion of the raw materials in the diet to the age of the birds (Ravindran and Hendriks, 2004) and the digestibility of the amino acids in the raw material itself (Lemme et al, 2004). Tarvid (1995) reported that digestion and absorption of dietary protein is influenced by age, however, there are contradictory reports on the effects of age on protein and amino acids digestibility in broiler chickens. Larbier and Chagneau (1992) reported increase in digestibility with age, Hakansson and Erikson (1974), Fonolla et al. (1981) reported a decline in digestibility with age. This discrepancy may be as a result of the contribution of amino acid of endogenous origin to the digesta. In order to take into account some of these factors that impact digestibility, diets are being formulated to include a safety margin. This procedure often leads to an increase in N and amino acid excretion which can result in increased environmental pollution as well as an increase in the cost of production.

The importance of MBM in poultry diets is found in its availability and its high CP contents (as high as 50%) (Angkanaporn, et al., 1996; Ravindran et al., 2002). Despite this high CP contents, MBM quality and digestibility across different MBM samples is not always uniform or predictable. Reasons for the variation in CP quality of MBM have been attributed to the sources as well as the processing techniques (Parsons et al., 1997; Wang and Parsons, 1998; Karakas et al., 2001). Due to the fact that MBM are sourced from different sources and processed under different conditions it is understandable that such may have different effects on their CP digestibility.

Establishing SIAAD for some raw materials used in poultry feeds at some ages will enable producers to be able to formulate diets based on digestible amino acid rather than on total amino acid. Hence, this will result in decreased safety margin and a reduction in N and amino acid excretion. The need and advantages of formulating diets based on digestible amino acid was discussed by Lemme et al. (2004).

The objective of this study was to determine apparent digestibility and SIAAD in chicks and turkey poults at two ages (d 5 and d 21) using NFD and CDP method to correct for basal IEAA flow.

MATERIAL AND METHODS

Meat and bone meal

Four meat and bone meal (MBM) samples from different sources were used in this study. The contents of MBM1 were all beef and that of MBM2 was all pork. Meat and bone meal 3 (MBM3) was from mix species while MBM4 was mix species plus grocery and parker trimmings.

Diet formulation

Two semi-purified diets were used as the control diets for the determination of basal endogenous amino acids whose values were used to standardize AID to obtain SIAAD. The control diets were a NFD and a diet containing 100 g casein/kg diet (CDP). The remaining four diets were formulated to contain about 20% crude protein (CP) with each of the four MBM samples supplying all the CP contents in the diets. Diet composition and the analyzed amino acid nutrient composition of the experimenting diets are reported in Tables 1 and 2, respectively. Two basal diets (a broiler starter diet and a turkey starter diet) were also made. Chromic oxide was added as the indigestible marker at 3 g/kg diet. Broiler and turkey poults were fed the same experimental diets but the studies were conducted at two different locations.

Birds and housing

One thousand three hundred and sixty eight 0-d old male broiler chicks (Ross 308, Aviagen, Huntsville, AL) and 1,368 male turkey poults (Nicholas) were obtained from commercial hatcheries. One thousand and eighty birds (1,080) from each species were weighed and randomly allocated to diets. These birds were placed on the experimental diets on d 0 and were euthanized on d 5.

Diets used for estimating IEAA and total amino acid (TAA) flow were semi-purified containing 0 or 100 g casein/kg diet. Casein supplied all the amino acids in these diets. The control diets and the diets containing the MBM samples were fed for 5 consecutive days before ileal contents collection. Six replicate cages containing 30 birds per cage were euthanized and the ileal contents removed by flushing with distilled water on d 5. The remaining birds were fed a conventional corn-soybean meal-based starter diet appropriate for the respective species until day 16 when 288 birds from each species were randomized to cages with 8 birds per cage. On day 21, the 288 were euthanized and ileal contents collected on day 21 as described for d 5. All birds were euthanized using CO_2 asphyxiation.

Birds were raised in battery cages (Alternative Design Manufacturing and Supply, Inc. Siloam Springs, AR) till the end of the study in an environmentally controlled room with 24-h of light. Room temperature was 35 °C during the first week and was dropped by 5 degrees during the subsequent weeks. Birds had free access to feed and water. All animal care procedures were approved by the Purdue University Animal Care and Use Committee.

Sampling and ileal digesta processing

Content of the ileum (portion of the small intestine from Meckel's diverticulum to about 5 mm proximal to the ileo-cecal junction) was flushed with distilled water. Ileal contents were flushed with distilled water using 50 cl syringes (d 5) and wash bottles (d 21). Ileal digesta from birds within a cage were pooled and stored in a freezer (-20 °C) until they were processed. Samples were freeze-dried, ground using mortar and pestle, and were sent to the University of Missouri Experimental Station and Chemical Laboratory for complete amino acid profile and chromium analysis.

Chemical analysis

Prior to diet formulation, the crude protein, N x 6.25, (%), ash (%) and the peroxide values (meq/kg) of the MBM samples were determined on air dry basis. In addition to this, the hydroxyproline contents (%) of the diets were determined (see details under the result section). Dry matter content was determined on ground diets and ileal digesta by drying the samples at 100 °C for 24 h. Amino acids and chromium analyses were conducted at the University of Missouri Experiment Station and Chemical Laboratory. For amino acid analyses, samples were hydrolyzed in a 6 *N* HCl for 24 h at 110 °C under N atmosphere. For sulfur containing amino acids, methionine and cysteine, performic acid oxidation was carried out before acid hydrolysis. For tryptophan analysis, samples were hydrolyzed using barium hydroxide. The amino acids in the hydrolysate were then determined by HPLC after postcolumn derivatization (AOAC, 200; 982.30 E [a, b, c]. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. Chromium was determined by the inductively coupled plasma atomic emission spectroscopy method (AOAC, 2000; 990.08) following nitric/perchloric acids wet ash digestion. Nitrogen in the MBM samples was determined by the combustion method (AOAC, 2000; 990.03) (model FP2000, LECO Corp., St. Joseph, MI) using EDTA as a standard.

Calculations

Ileal EAA and TAA flow from both species was calculated as mg of amino acid and TAA flow per 1 kg of feed on DM intake (DMI) basis using the following formula (Moughan et al. 1992):

Ileal (basal) amino acid flow (mg/kg DMI) =

[amino acid in ileal digesta, mg/kg] x (diet chromium, mg/kg)/(ileal chromium, mg/kg)).

Standardized ileal amino acid digestibility, SIAAD, (%), was calculated using the formula given by Lemme et al. (2004):

Standardized ileal amino acid digestibility, SIAAD, (%) = Apparent digestibility (%) + ((basal IEAA flow in g/kg DMI)/(AA content of the raw material in g/kg DM) x100

Statistical analysis

Data were analyzed using the GLM Procedure of SAS (SAS Inst., Inc., Cary, NC). When the F-ratio is significant, treatment means were separated using Duncan multiple range test. The level of significance was set at $P < 0.05$.

RESULTS

The crude protein, ash, and hydroxyproline contents of the MBM samples (on air dry basis) were 45.5, 33.5, and 11.0 % (MBM1, all beef); 55.5, 23.7, and 12.3 % (MBM2, all pork); 54.7, 22.1, and 6.8 % (MBM3, mix species), 53.3, 24.2, and 6.1 % (MBM4, mix species plus grocery trimmings). The level of peroxide in the diets containing the MBM samples (on air dry basis) were 4.3, 109.7, 20.3, and 16.4 meq/kg of diet for MBM1, MBM2, MBM3, and MBM4, respectively. The analyzed Cp contents of the diets were 17.7, 20.9, 17.0, and 17.9 % for MBM1, MBM2, MBM3, and MBM4, respectively.

Apparent ileal digestibility (AID) on d 5 and 21 for broiler chicks when fed diets containing MBM from four different sources are reported in Table 3. For MBM2 and MBM3 the AID for DM and all the amino acids except for tryptophan and hydroxylysine were higher $(P < 0.05)$ on d 21 compared to AID on d 5. For MBM4, AID for most of the amino acids was not significantly different at both ages except for isoleucine, leucine, methionine, phenylalanine, threonine, valine, alanine, glutamic acid, and tyrosine with higher ($P < 0.05$) AID on d 21. Apparent digestibility values for TAA increased from d-5 to d-21 by 17.5% (MBM2), 17.7% (MBM3), and 9.5% (MBM 4). However, AID for MBM1 decreased by 11% from d-5 to d-21.

Table 4 shows the SIAAD of amino acids in broiler chicks when correction was by either NFD or CDP method for MBM1 and MBM2. On d-5, the SIAAD was higher (MBM1, $P < 0.05$) when correction was made by CDP method. However, there was no significant difference between the two methods of correction when TAA digestibility was measured. On d-21, however, there was no difference in SIAAD except for isoleucine, glutamic acid, and serine whose SIAAD was higher (MBM1, $P < 0.05$) when correction was by CDP method. The mean difference between the two methods of correction for TAA was 4.2% on d 5 and 2.6% on d 21.

Standardized ileal amino acid digestibility when correction was by either NFD or CDP method for MBM2 is also shown in Table 4. On d 5, there was no significant difference in SIAAD between the two methods. Isoleucien, valine, glutamic acid, and serine's SIAAD was higher (MBM2, $P < 0.05$) with CDP correction on d 21. The difference in TAA SIAAD between the two methods of correction was 4.0% (d 5) or 2.1% (d 21) (Table 4, MBM2). On d 5 (Table 5, MBM3), none of the amino acids showed any significant difference irrespective of the method of correction. On d 21, however, isoleucine, valine, glutamic acid, and serine were the amino acids whose SIAAD was higher (MBM3, $P < 0.05$) when standardization was by CDP method. For TAA the difference between the SIAAD between the two methods was 4.7% (d 5) and 2.7% (d 21).

The SIAAD for MBM4 is reported in Table 5. There was no significant difference in SIAAD between the two methods of standardization. On d 21, however, Isoleucien, glutamic acid, and serine's SIAAD was higher (MBM4, $P < 0.05$) with CDP correction on d 21. The difference between the two methods of standardization for TAA on d 5 and 21 were 4.2% and 2.8%, respectively.

Table 6 contains values of amino acids AID for the four MBM samples in turkey poults. Total amino acid AID for MBM1 was 60.5% (d 5), 61.2% (d21); MBM2, 78.0% (d 5), 82.0% (d 21), MBM3, 72.0% (d 5), 70.8% (d 21) and was 74.8% (d5) and 80.2% (d 21) for MBM4. Out of all the MBM samples used in this study, only histidine showed a significant improvement in digestibility with age. The difference in digestibility between d 5 and 21 for histidine for MBB1, MBM2, MBM3, and MBM4 were 28.4, 42.3, 30.7, and 35.8%, respectively. The difference in TAA digestibility between d 5 and 21 for MBB1, MBM2, MBM3, and MBM4 were 0.7%, 4.0%, 1.2% (a decrease) and 5.4%, respectively.

Standardized ileal amino acid digestibility of MBM1 and MBM2 in poults on d 5 are shown in Table 7. With either NFD or CDP as the standardization methods, there was no significant difference in SIAAD for all the amino acids on d 5 or d 21. The SIAAD for methionine, and threonine on d 5 were 63.8% (NFD) or 69.8% (CDP) and 58.8% (NFD) or 66.8% (CDP) for MBM1, respectively. On d 21 the SIAAD for methionine and threonine were 70.2% (NFD) or 78.0% (CDP) and 61.4% (NFD) or 73.48% (CDP) for MBM1, respectively. Standardization using CDP method resulted in an increase of 4.2% (d 5) or 6.0% (d 21) relative to when standardization was by NFD method.

Standardization of AID in poults (MBM2) using CDP resulted in a higher ($P < 0.05$) SIAAD values for isoleucine (77.6%- NFD, 84.2%- CDP), threonine (77.4%- NFD, 84.4%- CDP), valine (78.4%- NFD, 84.6%- CDP), and glutamic acid (82.4%- NFD, 87.6%- CDP) on d 5 (Table 7). On d 21, method of standardization did not result in any significant difference in digestibility for any of the amino acids. The differences in SIAAD for the standardization techniques for TAA were 4% and 6.0%, respectively for d 5 and 21.

Standardization of digestibility for MBM3 is shown in Table 8.The CDP method resulted in a higher (MBM3, $P < 0.05$) SIAAD for all the amino acids and TAA except for histidine. The SIAAD for TAA was 76.8% (NFD) and 81.2% (CDP). On d 21 there was no significant effect of standardization methods on ileal digestibility of all the amino acids. The difference in SIAAD for the standardization techniques for TAA was 2.6% and 6.0%, respectively for d 5 and 21.

The SIAAD in poults fed diet containing MBM4 is presented in Table 8. There was no significant difference in digestibility for all the amino acids irrespective of the standardization method used (MBM4). On d 21, method of standardization did not result in any significant difference in digestibility of all the amino acids. Numerically, CDP method resulted in about 4.0% (d 5) and 5.6% (d 21) increase in TAA digestibility.

DISCUSSION

The objective of this study was to determine and compare the effects of age (d 5 vs. d 21) and standardization methods (NFD vs. CDP) on apparent ileal digestibility and SIAAD values of MBM from four sources fed to broiler chicks and turkey poults. Accurate determination of amino acid digestibility in feed ingredients is essential by accounting for amino acid of endogenous origin in the digesta. Basal endogenous amino acids have been determined using the classical methods (NFD, CDP, the regression methods, and in fasted cockerels) in chickens (Sibbald, 1979; Okumura et al., 1981; Ravindran and Bryden, 1999) and in swine (de Lange et al, 1989; Fan et al., 1995). The MBM samples used in this study showed considerable variations in the level of their chemical composition. This variation in composition aggress with what have been reported earlier (Ravindran et al., 2002; Adedokun and Adeola, 2005) and is attributable to the fact that MBM are from different species, sourced from different rendering plants and are processed using different processing techniques.

Correcting the AID for IEAA flow in 5-wk-old broilers has been shown to have resulted in a relatively higher digestibility values (Angkanaporn et al., 1996). A number of studies have been conducted to determine (IEAA) and TAA flow in chicks and chickens (Ravindran and Hendrikcs, 2004; Ravindran et al., 2004).

Apparent ileal amino acid digestibility values in three (MBM2, MBM3, and MBM4) of the four MBM samples showed similar trends with an increase in AID with age. All the amino acids with the exception of tryptophan and hydroxylysine showed significant improvement with age (MBM2 and MBM3). For MBM from the mix species plus grocery trimmings (MBM4), hydroxylysine and hydroxyproline do not show any improvement with age. In addition to this, only isoleucine, leucine, methionine, phenylalanine, threonine, valine, alanine, glutamic acid, and tyrosine showed improvement in digestibility with age. The digestibility of MBM1 (all beef) is completely different from the other three MBM samples. This observation is difficult to explain. However, AID decreased with age for the all beef MBM in chicks. The greatest improvement with age in lysine and methionine was seen in MBM2 (17 and 25%) and MBM3 (16 and 25%). It is interesting to note that the high level of peroxide in the all pork MBM (MBM2) seems not to have any negative effect on its AID. In the same vein, the high level of hydroxyproline in the diets containing all beef and all pork appear not to have influenced apparent ileal amino acid digestibility. This observation is different from what is expected. Overall, amino acids AID for all the MBM samples on d 21 was similar to the mean AID values for 19 MBM samples reported by Ravindran et al. (2002).

The AID values on d 21 are lower that what Huang et al. (2005) reported for 14 and 21-d-d old broilers fed diet containing 20% CP from MBM. Also, the AID values for lysine, methionine, threonine, as well as some other amino acids in this study was higher than what Angkanaporn et al. (1996) reported for 5-wk-old broilers. The processing methods and the source of the MBM may have contributed to the observed differences in AID (Shirley and Parsons, 2000; Karakas et al., 2001).

Apparent ileal amino acid digestibility in poults with the exception of histidine was not influenced by age. With the exception of the all beef MBM on d 5, poults AID was higher than that of the chicks at both ages. The lack of a difference in poults' AID at both ages could be due to species effects or as a result of the method of ileal collection used. Ileal contents in poults were collected by gently squeezing out the digesta as against flushing with distilled water as done in chicks. This may have resulted in sloughing off of more epithelial cells into the digesta as a result of the applied pressure, especially on d 21 when the birds are bigger and with greater intestinal surface area. Similar trends were also seen in the ileal endogenous amino acids used for the correction. The ileal endogenous amino acids used for standardization in chicks was similar to values obtained in an earlier study conducted in our lab, however, values from the poult in this study were different from values obtained from earlier studies in our lab (unpublished data).

The overall amino acid AID was higher (numerically) in poults for all the MBM except for the all beef MBM sample where digestibility at d 5 for chicks was higher than for poults. This is an indication that turkey poults are better able to digest amino acids in MBM at both ages (d 5 and 21) than chicks. Histidine is an amino acid that stood out from the remaining amino acids on d 5 in poults. On d 5, the digestibilities of histidine was significantly lower that on d 21. This difference between d 5 and 21 was as high as 41%. Hydroxyproline and hydroxylysine digestibility was relatively higher in poults than in chicks. Unlike in poults the digestibility for these two amino acids had high standard deviation in chicks which is an indication of the extent of variation in digestibility between species or across experimental locations. This result shows that in most cases, age and the level of hydroxyproline and hydroxylysine do not have a significant impact on their digestibility. Hydroxyproline from porcine MBM seems to be better digested by poults than other sources of MBM used in this study.

Standardization of the AID values resulted in a relatively higher digestibility values for all the amino acids in the MBM samples evaluated. However, unlike what Angkanaporn et al. (1996) obtained in their study where the standardized values for all the amino acids were similar (about 73%) the SIAAD values obtained from our study were different for each of the amino acids. This difference between these two studies may be as a result of the standardization method. Angkanaporn et al. (1996) used the homoarginine method where homoarginine digestibility is considered as being representative of the standardized values for all amino acids within an ingredient. This assumption may not always hold. For the broiler chicks, standardization using CDP resulted in significantly higher SIAAD for isoleucine, glutamic acid, and serine for all the MBM samples (chicks) or isoleucine and glutamic acid for MBM2 (poults). This shows that SIAAD for these amino acids in MBM will be method sensitive.

When the two methods of corrections were compared within each species and age, it was discovered that with age there was a decrease in the difference in SIAAD between the two methods from 4.3% (d 5) to 2.6% (d 21) for chicks and 4.2% (d 5) and 5.7% (d 21) for poults. This means that either of the two methods of correction for basal IEAA flow will be good for standardizing amino acid AID at older ages than at younger ages in chicks while the opposite is true for poults.

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Table 1. Dietary composition of the experimental diets (on as is basis)

Ingredient g/kg	N-F-D	10% casein	MBM 1	MBM 2	MBM 3	MBM 4
Corn starch	408	308	0	79	74	64
Dextrose	401	401	401	401	401	401
Casein	0	100	0	0	0	0
MBM	0	0	440	361	366	376
Solkafloc	50	50	50	50	50	50
Soy oil	50	50	50	50	50	50
Vitamin-Mineral Premix[1]	5	5	5	5	5	5
Dicalcium phosphate	19	19	-	-	-	-
NaHCO3	20	20	20	20	20	20
KCl	12	12	12	12	12	12
MgO	2	2	2	2	2	2
Choline chloride	3	3	3	3	3	3
Limestone	13	13	-	-	-	-
NaCl	2	2	2	2	2	2
Chromic Ox Premix[2]	15	15	15	15	15	15
Total	1000	1000	1000	1000	1000	1000

[1]Provided per kg of diet: Iron, 71.6 mg; Copper, 11.0 mg; Manganese, 178.7 mg; Zinc, 178.7 mg; Iodine, 3.0 mg; Selinium, 0.4 mg. Vitamin A (retinyl acetate), 18,904.3 IU; Vitamin D_3 (cholecalciferol), 9,480.0 IU; Vitamin E (DL-α-tocopheryl acetate), 63.0 IU; Vitamin K activity, 6.4 mg; Thiamine, 3.2 mg; Riboflavin, 9.4 mg; Pantothenic acid, 34.7 mg; Niacin, 126.0 mg; Pyridoxine, 4.7 mg; Folic acid, 1.6 mg; Biotin, 0.5 mg; Vitamin B_{12}, 35.4 mcg; choline, 956.9 mg.

[2]Chromic oxide (Cr_2O_3) premix added as index at a ratio 1:4 of chromic oxide:corn starch.

Table 2. Analyzed amino acids and nitrogen composition of the experimental diets (on as is basis)

	Diet[1]					
Item	NFD[2]	10% Casein	MBM1	MBM2	MBM3	MBM4
Essential amino acids, g/kg						
Arginine	0.00	3.00	12.70	15.10	11.20	12.10
Histidine	0.00	2.50	2.90	3.90	4.40	4.30
Isoleucine	0.00	4.50	4.60	5.70	5.80	6.60
Leucine	0.10	8.30	10.30	12.50	12.30	13.30
Lysine	0.10	6.90	9.60	11.00	10.80	12.20
Methionine	0.00	2.21	2.08	2.89	2.78	3.45
Phenylalanine	0.00	4.50	5.90	7.10	6.70	7.20
Threonine	0.00	3.30	5.30	6.80	6.30	6.90
Tryptophan	<0.40	1.00	1.10	1.10	1.10	1.00
Valine	0.10	5.70	7.20	8.30	8.10	8.50
Nonessential amino acids, g/kg						
Alanine	0.10	2.60	13.90	15.80	12.40	12.50
Aspartic acid	0.10	6.00	12.70	16.00	14.00	15.20
Cysteine	0.00	0.32	1.46	1.66	1.73	1.78
Glutamic acid	0.10	19.00	20.90	25.60	22.90	23.80
Glycine	0.00	1.60	27.20	29.80	18.70	18.30
Hydroxyproline	0.00	0.00	11.00	12.30	6.80	6.10
Proline	0.10	9.00	15.80	18.20	12.20	11.80
Serine	0.00	4.00	6.10	7.50	5.90	6.70
Tyrosine	0.00	3.90	3.60	4.40	4.00	4.70
Total amino acids	1.80	89.30	176.84	208.60	170.41	178.63

[1]MBM = Meat and bone meal
MBM1, from all beef, contained 45.50% CP
MBM2, from all pork, contained 55.49% CP
MBM3, mix species, contained 54.73% CP
MBM4, mix species plus some grocery trimmings, contained 53.30% CP
[2]Nitrogen-free diet

Table 3. Apparent ileal amino acid and total amino acid digestibility in chicks fed meat and bone meal from four different sources (on DM basis, days 5 and 21)

	Apparent digestibility, %[1]											
	MBM1			MBM2			MBM3			MBM4		
Age, days	5	21		5	21		5	21		5	21	
Item	C	C	SD[2]	D	D	SD[2]	E	E	SD[2]	F	F	SD[2]
N[3]	6	6		4	6		6	6		5	6	
DM	67.50^a	55.17^b	5.257	50.00^b	65.67^a	5.802	55.67^b	69.83^a	5.312	59.00	65.17	6.983
Essential amino acid												
Arginine	77.67^a	65.00^b	4.993	70.50^b	81.83^a	3.198	62.50^b	76.33^a	4.763	66.20	72.67	5.479
Histidine	71.33	67.50	4.133	47.25^b	68.50^a	5.434	46.17^b	63.17^a	6.691	58.80	68.67	7.228
Isoleucine	65.50	61.33	4.635	41.25^b	66.00^a	5.108	42.50^b	63.83^a	6.390	54.80^b	68.33^a	7.790
Leucine	71.17	66.83	4.021	52.25^b	73.17^a	4.711	50.00^b	69.33^a	6.240	60.20^b	71.67^a	6.881
Lysine	76.00^a	68.83^b	3.779	55.50^b	72.67^a	4.188	52.83^b	68.83^a	5.456	63.80	72.00	6.332
Methionine	68.50	65.50	4.889	48.75^b	73.33^a	5.269	44.50^b	67.50^a	7.204	62.00^b	72.83^a	7.218
Phenylalanine	70.67	66.50	4.109	54.25^b	74.33^a	4.185	50.33^b	69.50^a	5.923	59.00^b	71.00^a	7.087
Threonine	62.33^a	54.50^b	5.611	39.50^b	64.17^a	7.157	37.17^b	59.33^a	8.719	50.20^b	62.67^a	8.870
Tryptophan	81.50	77.67	3.699	64.75	69.00	4.074	72.33	69.17	2.533	67.20	65.17	6.828
Valine	67.50	65.83	4.103	45.25^b	70.50^a	7.667	46.33^b	67.67^a	6.638	53.60^b	68.83^a	8.512
Nonessential amino acid												
Alanine	74.83^a	64.67^b	4.798	65.25^b	79.83^a	3.866	55.83^b	73.67^a	5.746	60.40^b	70.00^a	6.825
Aspartic acid	66.50^a	55.50^b	5.320	37.25^b	58.33^a	6.893	31.00^b	51.33^a	8.794	42.60	50.33	10.814
Cysteine	34.33^a	17.17^b	9.748	-11.75^b	21.83^a	12.726	19.67^b	39.67^a	10.967	15.60	28.67	16.433
Glutamic acid	72.67^a	63.17^b	4.606	56.25^b	74.00^a	4.780	51.50^b	68.50^a	5.925	59.00^b	68.83^a	7.093
Glycine	75.17^a	60.83^b	5.307	67.50^b	79.67^a	4.158	57.17^b	72.33^a	6.182	56.00	62.67	7.493
Hyrdoxylysine	69.00^a	55.17^b	5.393	88.50	84.33	19.622	65.50	75.83	27.431	43.00	54.83	20.273
Hyrdoxyproline	72.67^a	44.33^b	8.722	64.25^b	76.67^a	6.084	52.50^b	65.67^a	8.572	49.40	53.67	10.288
Proline	71.00^a	54.00^b	5.586	60.00^b	74.17^a	5.010	49.83^b	66.83^a	6.555	51.00	58.83	8.900
Serine	64.17^a	49.50^b	6.406	44.50^b	64.83^a	7.809	36.67^b	57.67^a	9.730	48.60	59.00	9.541
Tyrosine	66.00^a	59.00^b	5.422	44.00^b	67.17^a	6.213	41.17^b	62.67^a	6.886	56.20^b	67.00^a	7.709
Total amino acid	70.50^a	59.50^b	5.206	54.50^b	72.00^a	5.037	48.33^b	66.00^a	6.445	55.20	64.67	7.875

[1] Means within the same row and within the same treatment with different superscripts are significantly different, $P < 0.05$.; MBM= Meat and bone meal
[2] Standard deviation.
[3] Number of replicates. Each cage represents an experimental unit with 30 birds per cage at day 5 and 8 birds per cage at day 21.

Table 4. Standardized ileal amino acid and total amino acid digestibility s in chicks fed meat and bone meal from beef and pork[1].

	MBM1 (all beef)						MBM2 (all pork)					
Age, days	5			21			5			21		
Method	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6		4	4		6	6	
Essential amino acid												
Arginine	81.0	82.7	3.81	67.5	68.0	5.96	73.5	75.0	4.56	83.8	84.7	2.15
Histidine	79.0^{b}	85.2^{a}	3.05	72.5	75.2	5.08	53.3	57.8	7.83	72.0	74.0	3.69
Isoleucine	74.7^{b}	85.5^{a}	3.51	67.7^{b}	76.7^{a}	5.72	48.5	57.5	7.94	71.2^{b}	78.3^{a}	3.10
Leucine	77.8^{b}	82.8^{a}	3.06	70.8	72.5	4.72	57.8	61.8	6.85	76.5	78.0	2.608
Lysine	81.0^{b}	86.0^{a}	2.53	72.7	75.5	4.70	59.8	64.5	5.35	75.8	78.3	3.07
Methionine	75.8^{b}	82.7^{a}	3.17	69.5	74.5	6.22	54.5	59.3	7.83	76.3	79.8	3.07
Phenylalanine	77.3^{b}	81.7^{a}	2.70	71.0	71.5	5.13	60.3	63.5	5.97	78.2	78.5	2.42
Threonine	73.7^{b}	81.3^{a}	4.03	62.7	66.0	6.55	48.3	54.0	10.62	70.5	73.2	4.08
Tryptophan	NA	NA	NA	NA	83.2	NA	NA	NA	NA	NA	74.7	NA
Valine	75.3^{b}	82.2^{a}	3.41	70.5	75.0	4.71	52.0	58.0	11.92	74.5^{b}	78.5^{a}	3.02
Nonessential amino acid												
Alanine	78.2	80.3	3.74	66.8	68.0	5.58	68.3	70.3	5.56	81.7	82.8	2.24
Aspartic acid	72.8	78.2	4.17	59.8	62.8	6.11	42.5	46.5	10.12	61.7	64.2	4.58
Cysteine	51.0	56.0	7.38	30.0	31.2	11.65	3.0	7.3	18.67	33.2	34.3	6.87
Glutamic acid	78.2^{b}	86.3^{a}	3.35	67.0^{b}	74.2^{a}	5.37	60.5	67.5	7.00	77.0^{b}	82.7^{a}	2.71
Glycine	77.2	77.8	4.62	61.8	62.0	5.94	69.0	69.5	6.01	80.7	80.7	2.50
Proline	74.3	77.5	5.09	56.8	58.5	6.58	62.8	65.8	7.63	76.7	78.2	2.76
Serine	73.2^{b}	83.2^{a}	4.88	55.5^{b}	65.5^{a}	7.64	52.0	60.0	11.86	70.0^{b}	78.0^{a}	4.29
Tyrosine	74.7^{b}	81.7^{a}	3.78	65.0	66.5	6.63	51.0	56.8	8.86	72.3	73.3	3.20
Total amino acid	76.0	80.2	3.73	63.2	65.8	6.01	58.8	62.8	7.63	75.2	77.3	3.00

[1] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[2] Standard deviation.
[3]Nitrogen-free diet
[4]Number of replicates. Each cage represents an experimental unit with 30 birds per cage

Table 5. Standardized ileal amino acid and total amino acid digestibility s in chicks fed meat and bone meal from mix species and plus grocery trimmings[1].

	MBM3 (mix species)						MBM4 (mix species plus grocery trimmings)					
Age, days	5			21			5			21		
Method	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	6	6		6	6		5	5		6	6	
Essential amino acid												
Arginine	66.5	68.5	6.50	79.2	79.3	1.74	69.8	71.4	7.09	75.5	75.7	4.16
Histidine	51.2	55.0	9.12	66.8	68.2	2.79	63.8	67.8	9.86	71.8	73.7	4.08
Isoleucine	49.5	58.2	8.57	68.8[b]	75.8[a]	3.06	60.8	68.8	10.96	72.5[b]	78.7[a]	3.56
Leucine	55.5	59.5	8.07	72.8	74.0	2.30	65.2	69.2	9.58	75.0	76.0	3.52
Lysine	57.3	62.0	7.22	72.0	74.5	2.27	67.4	71.8	8.46	74.7	76.7	3.45
Methionine	50.2	55.2	9.68	70.7	74.2	2.97	66.4	70.4	9.94	75.3	78.3	4.27
Phenylalanine	56.3	60.0	7.97	73.5	73.8	2.37	65.0	68.0	9.87	74.8	75.0	3.59
Threonine	46.5	52.8	11.77	66.3	69.0	3.22	58.6	64.4	12.41	69.2	71.5	5.16
Tryptophan	NA	NA	NA	NA	74.8	NA	NA	NA	NA	NA	71.2	NA
Valine	53.2	59.2	9.02	71.8[b]	75.7[a]	2.45	60.2	65.8	12.08	72.8	76.3	3.64
Alanine	60.8	63.3	7.68	76.2	77.2	1.72	64.2	66.4	8.93	72.5	73.8	4.32
Aspartic acid	36.8	41.8	12.19	55.3	58.0	3.57	48.4	52.4	14.42	54.0	56.5	6.31
Cysteine	33.7	37.7	14.42	50.5	51.5	5.65	29.2	33.2	22.53	39.2	40.7	7.90
Glutamic acid	56.5	63.8	7.99	72.0[b]	78.3[a]	2.39	63.8	71.0	9.77	72.2[b]	78.3[a]	4.08
Glycine	59.7	61.0	8.56	74.2	74.7	2.37	58.4	59.8	9.45	64.7	65.5	5.85
Proline	54.3	58.3	8.89	70.3	72.5	2.66	55.4	59.8	11.17	62.7	64.8	6.67
Serine	45.7	55.7	13.13	64.2[b]	74.2[a]	4.49	56.6	65.4	12.93	65.0[b]	73.8[a]	5.77
Tyrosine	48.7	55.2	9.42	67.8	69.0	2.16	62.6	68.2	10.82	71.8	72.8	4.26
Total amino acid	53.8	58.5	8.97	70.0	72.7	2.22	60.6	64.8	10.50	68.2	71.0	4.64

[1] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[2] Standard deviation.
[3]Nitrogen-free diet
[4]Number of replicates. Each cage represents an experimental unit with 30 birds per cage

Table 6. Apparent ileal amino acid and total amino acid digestibility in turkey poults fed meat and bone meal from four different sources (on DM basis, days 5 and 21)

	Apparent digestibility, % [1]											
	MBM1			MBM2			MBM3			MBM4		
Age, days	5	21		5	21		5	21		5	21	
Item	C	C	SD[2]	D	D	SD[2]	E	E	SD[2]	F	F	SD[2]
N[3]	4	5		5	4		5	4		5	5	
DM	53.25	55.00	8.235	75.00	74.50	5.843	75.00	72.25	4.013	71.40	76.40	3.715
Essential amino acid												
Arginine	65.50	65.00	6.876	83.60	86.50	4.212	77.60	76.50	5.659	79.60	84.40	4.037
Histidine	43.25[b]	74.40[a]	10.730	38.00[a]	80.25[b]	14.257	41.80[b]	72.50[a]	8.617	41.40[b]	77.20[a]	6.083
Isoleucine	55.75	61.40	10.914	70.20	75.00	8.007	65.60	65.25	9.471	74.00	81.00	6.205
Leucine	60.50	64.20	9.364	75.20	79.50	6.738	70.40	70.25	7.653	75.00	81.80	5.418
Lysine	60.50	65.00	9.494	75.80	80.25	5.899	72.00	71.25	6.400	74.80	80.80	5.263
Methionine	56.75	63.00	11.242	72.60	78.50	6.992	66.50	67.50	8.460	73.80	81.40	6.727
Phenylalanine	59.25	63.00	9.847	74.80	79.00	6.916	70.20	69.00	8.356	73.60	81.00	5.992
Threonine	48.75	49.60	14.352	68.60	72.00	9.344	64.00	59.50	11.155	68.80	76.00	6.917
Tryptophan	66.20	69.20	15.085	82.20	78.25	8.329	74.40	64.50	17.494	79.20	85.40	4.848
Valine	57.00	64.40	8.44	70.80	78.00	7.219	68.80	69.75	7.815	73.00	80.80	5.532
Nonessential amino acid												
Alanine	67.50	67.00	6.199	82.60	86.25	3.890	77.60	77.75	4.810	78.80	84.40	4.062
Aspartic acid	54.50	55.20	10.225	71.40	74.75	7.211	66.40	63.00	8.858	69.00	72.40	6.607
Cysteine	33.00	32.40	15.124	43.60	47.75	15.306	49.40	40.75	15.371	47.80	54.00	11.151
Glutamic acid	61.25	62.40	7.892	77.80	82.00	5.409	72.40	71.75	6.761	75.40	80.80	5.244
Glycine	69.00	66.00	6.188	86.40	89.75	2.328	82.20	82.50	2.971	80.40	83.80	2.958
Hyrdoxylysine	64.75	55.20	6.542	85.20	86.50	3.019	83.40	77.75	3.999	81.60	78.20	3.041
Hyrdoxyproline	69.25	62.00	8.724	90.00	92.75	2.228	86.60	88.25	2.827	83.60	84.00	3.450
Proline	64.25	60.80	6.563	84.20	87.00	3.439	78.00	77.50	4.424	78.60	82.40	3.578
Serine	48.00	46.60	13.655	71.60	74.50	8.571	64.00	61.00	10.406	65.60	72.600	7.470
Tyrosine	52.75	57.00	13.590	72.80	76.75	7.978	67.80	67.00	8.757	71.60	79.40	6.269
Total amino acid	60.50	61.20	7.651	78.00	82.00	5.345	72.00	70.75	6.684	74.80	80.20	5.020

[1] Means within the same row and within the same treatment with different superscripts are significantly different, $P < 0.05$.; MBM= Meat and bone meal
[2] Standard deviation; [3] Number of replicates. Each cage represents an experimental unit with 30 birds per cage at day 5 and 8 birds per cage at day 21.

Table 7. Standardized ileal amino acid and total amino acid digestibility s in turkey poults fed meat and bone meal from beef and pork[1].

	MBM1 (beef)						MBM2 (pork)					
Age, days	5			21			5			21		
Method	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	4	4		5	5		5	5		4	4	
Essential amino acid												
Arginine	68.8	71.3	4.82	68.4	71.8	8.23	86.6	89.2	2.40	89.5	92.8	5.77
Histidine	53.8	59.5	15.07	74.4	74.4	5.94	48.4	54.4	15.34	80.3	80.3	12.29
Isoleucine	63.8	70.8	6.65	69.4	79.4	13.24	77.6[b]	84.2[a]	3.54	82.8	92.3	11.34
Leucine	66.8	71.5	5.71	70.6	77.2	11.50	80.6	85.2	3.35	85.5	91.3	9.29
Lysine	67.5	73.3	5.29	72.4	79.8	12.08	81.2	85.6	3.28	86.3	92.0	8.53
Methionine	63.8	69.8	6.24	70.2	78.0	13.90	79.0	84.0	3.94	84.8	91.8	9.50
Phenylalanine	66.3	70.5	6.05	69.8	76.4	12.21	81.2	85.2	3.49	85.5	91.5	9.95
Threonine	58.8	66.8	6.99	61.4	73.4	17.81	77.4b	84.4a	4.51	82.0	92.0	13.37
Tryptophan					88.2		N/A	N/A	N/A	N/A	97.3	N/A
Valine	64.8	71.0	5.90	72.0	80.6	9.84	78.4b	84.6a	4.13	85.0	94.0	9.90
Alanine	70.5	73.3	4.86	70.0	73.6	7.01	86.0	88.2	2.47	89.8	93.0	5.37
Aspartic acid	60.5	66.0	6.52	62.0	69.0	12.19	77.0	81.8	3.59	80.8	87.5	10.25
Cysteine	46.3	54.0	10.53	48.2	62.4	17.79	59.6	69.2	7.16	66.0	83.8	21.97
Glutamic acid	66.3	72.3	5.38	67.4	74.6	9.29	82.4[b]	87.6[a]	3.01	86.8	93.0	7.43
Glycine	70.8	72.0	6.50	67.6	69.0	7.34	88.4	89.4	2.35	91.5	93.5	3.42
Proline	67.3	70.0	5.30	64.0	68.0	7.71	87.0	89.6	1.98	90.0	94.0	4.55
Serine	56.0	64.0	6.48	56.0	68.6	17.25	79.0	85.6	4.63	82.5	93.5	12.07
Tyrosine	60.8	66.5	6.76	65.6	73.8	16.80	79.6	84.4	3.94	84.3	91.0	11.35
Total amino acid	65.3	69.5	5.68	66.8	72.8	8.87	82.8	86.8	3.11	87.0	93.0	7.44

[1] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[2] Standard deviation.
[3]Nitrogen-free diet
[4]Number of replicates. Each cage represents an experimental unit with 30 birds per cage

Table 8. Standardized ileal amino acid and total amino acid digestibility s in turkey poults fed meat and bone meal from mix species and plus grocery trimmings [1].

	MBM3 (mix species)						MBM4 (mix species plus grocery trimmings)					
Age, days	5			21			5			21		
Method	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]	NFD[3]	CDP	SD[2]
N[4]	5	5		4	4		5	5		5	5	
Essential amino acid												
Arginine	81.0^b	83.6^a	1.63	80.5	84.3	8.56	82.6	84.8	3.84	87.4	90.4	4.04
Histidine	52.4	58.0	9.79	72.5	72.5	6.66	52.4	58.2	7.62	77.2	77.2	4.15
Isoleucine	72.0^b	77.8^a	2.09	72.0	80.3	13.97	79.2	84.2	5.93	86.4	93.4	6.23
Leucine	75.4^b	79.4^a	2.07	75.3	80.3	11.44	79.6	83.0	5.51	86.2	91.2	5.54
Lysine	76.2^b	79.8^a	1.79	75.8	80.3	9.83	79.6	83.2	5.29	85.8	90.4	5.39
Methionine	71.4^b	76.4^a	2.88	72.8	79.3	12.45	78.4	82.4	6.99	86.4	91.4	6.15
Phenylalanine	75.4^b	79.4^a	2.07	74.5	80.3	12.23	79.0	82.6	5.84	86.0	91.4	5.99
Threonine	72.2^b	78.6^a	3.12	69.0	78.5	16.49	82.2	76.2	6.57	84.6	93.4	7.22
Tryptophan	NA	NA	NA	NA	81.8	NA	NA	NA	NA	NA	98.4	NA
Valine	75.4^b	81.0^a	2.21	76.0	83.8	11.65	79.2	84.4	4.53	86.8	93.8	6.10
Alanine	80.6^b	83.6^a	1.52	80.8	84.8	7.14	82.0	84.8	4.17	87.4	90.8	3.87
Aspartic acid	71.6^b	75.8^a	2.69	68.3	74.3	12.84	73.8	78.0	4.96	77.6	83.0	7.82
Cysteine	64.2^b	72.4^a	4.53	57.3	73.3	23.27	60.8	67.8	7.73	69.0	83.0	13.75
Glutamic acid	76.4^b	81.4^a	2.07	75.8	82.0	9.91	84.2	79.4	5.22	84.8	90.8	5.26
Glycine	84.2^b	86.2^a	0.84	84.8	87.0	4.22	82.6	84.4	2.66	86.2	88.2	3.49
Proline	81.6^b	85.0^a	1.18	81.5	86.3	6.43	81.6	84.8	3.39	85.8	90.6	4.03
Serine	72.0^b	79.2^a	4.14	69.8	81.8	14.73	73.0	80.0	7.35	80.8	92.0	8.02
Tyrosine	74.2^b	78.4^a	2.18	74.0	80.5	13.21	77.6	81.6	5.68	85.8	91.8	6.61
Total amino acid	76.8^b	81.2^a	1.72	76.8	82.8	10.05	79.0	83.0	4.42	85.4	91.0	5.26

[1] Means within the same row with different superscripts are significantly different, $P < 0.05$.
[2] Standard deviation.
[3] Nitrogen-free diet
[4] Number of replicates. Each cage represents an experimental unit with 30 birds per cage