Development and Evaluation of a New Precision-Fed Chick Assay for Determining Amino Acid Digestibility and Metabolizable Energy of Feed Ingredients

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Introduction

Approximately 70% of the cost of producing poultry meat and eggs is associated with feed costs and 90-95% of the feed costs are associated with meeting metabolizable energy (ME) and protein/amino acid (AA) requirements. Thus, nutritionists need accurate and reliable values for ME and digestible AA in feed ingredients. Consequently, rapid, accurate, and inexpensive assays are needed for determining ME and AA digestibility. These values are often determined using the 48-hour precision-fed rooster digestibility assay and there is concern that these values may not be accurate for younger broiler chickens. Consequently, considerable research has been conducted in the last 3-5 years to develop an ileal AA digestibility assay using ad libitum-fed broiler chicks. Although the ad libitum ileal method has been successful, it does have some limitations. It is considerably more expensive than the simple 48-hour precision-fed cecectomized rooster assay, it requires many more animals, it requires much more time to obtain results and it requires a much larger amount of feed sample since a large number of chicks are fed for at least four days. Also, the ad libitum chick assay is not suitable for determining ME of feed ingredients since the test feed ingredients are fed as a part of a semi-purified diet. The overall objective of this proposed study was to develop and evaluate a new precision-fed broiler chick assay for determining ME and AA digestibility of feed ingredients. The development of an accurate and rapid precision-fed chick assay will provide researchers and nutritionists with another tool or method for determining the extremely important ME and AA digestibility values.

Materials and Methods

Experiment 1-Determination of the Ileal Endogenous Amino Acid Losses for Precision-fed Chicks

The first experiment was conducted to determine endogenous amino acid losses so that standardized amino acid digestibility values could be calculated later. Twenty-four day-old broiler chicks (Ross 308) were placed on a nutritionally complete starter diet until day 21. On day 21, they were fasted overnight and then randomly allocated into 4 groups of 6 chicks. Each chick was then precision-fed 10 g of a nitrogen-free diet

(NFD). At four hours post-feeding, the chicks were euthanized via CO_2 inhalation and the ileal digesta were collected and pooled for the 6 chicks within each of the 4 replicate groups. The ileal digesta were then freeze-dried, ground and analyzed for amino acids and chromium at the Experiment Station Chemical Laboratories, University of Missouri-Columbia. The ileal endogenous amino acid flow (IEAA) was calculated as milligrams of amino acid per kilogram of feed dry matter intake (DMI) using the following formula:

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

The next series of experiments were conducted to determine and compare amino acid digestibility values among the standardized ileal amino acid chick assay, the precision-fed rooster assay and the new precision-fed chick assay. The feed ingredients evaluated were corn, DDGS, soybean meal, canola meal, corn gluten meal (CGM) and meat and bone meal (MBM).

Standardized Ileal Amino Acid Digestibility Chick Assay (SIAAD)

Male Ross 308 broiler chicks were obtained at 1 d of age from a commercial hatchery and fed a nutritionally complete starter diet until d 16. After overnight fasting, the birds were weighed individually and randomized to 5 dietary treatments, with 5 birds per pen, 4 replicate pens per experimental diet. The birds were fed the 5 experimental diets for a five day period. On d 21, birds were killed by CO_2 asphyxiation and ileal digesta were collected. The experimental diets were formulated to contain approximately 20% CP (with the exception of the corn diet, which was approximately 7% CP), with each of the feedstuffs supplying the entire CP in the diets. Chromic oxide was added to all diets as an indigestible marker at 0.30% of the diet, with all diets being fed in mash form.

Precision-fed cecectomized rooster assay (PFR)

Precision-fed rooster assays utilizing cecectomized Single Comb White Leghorn roosters were conducted. After 24 hours of feed withdrawal, four cecectomized roosters were tube fed approximately 30 grams of each feed ingredient. Excreta were then quantitatively collected for 48 hours. Endogenous corrections for amino acids were made using excreta from roosters that had been fasted for 48 hours.

Precision-fed ileal amino acid chick assay (PFC)

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

hours after feeding, the chicks were euthanized via CO_2 asphyxiation and ileal digesta were collected.

Sampling, Ileal Digesta, and Excreta Processing

For the SIAAD and the PFC, the contents of the ileum were considered to be the part of the small intestine from the Meckel's diverticulum to the approximately 1 cm proximal to the ileo-cecal junction. The ileal digesta from birds within pens or groups were pooled, frozen, and stored at -20ºC until they were processed. For the rooster assay, the excreta were also frozen and stored at -20ºC until processing. All ileal and excreta samples were freeze-dried, ground by using a mortar and pestle and analyzed amino acids.

Calculations

Amino acid digestibility for the SIAAD and PFC were calculated using the formulas below. DMI is dry matter intake.

APPARENT ILEAL AMINO ACID DIGESTIBILITY =

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

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =

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

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

STANDARDIZED AMINO ACID DIGESTIBILITY, %=
[(Amino acid in feed ingredient (mg) − Amino acid excreta (mg) + endogenous amino acid (mg))/ amino acid in feed ingredient (mg)] × 100.

Results and Discussion

The results of the determination of endogenous amino acid losses in Experiment 1 indicated that the values in the PFC were higher than those previously reported for ad libitum fed chicks. The reason for the higher PFC values is unknown but may be due to the low feed intake of the PFC chicks and/or that the PFC were fasted prior to tube feeding the nitrogen free diet.

When the corn sample was fed in the three assays, the PFC generally yielded numerically higher amino acid digestibility coefficients with the difference being

significant ($P<0.05$) for some amino acids (Table 1). The increased amino acid digestibility may be due to a high endogenous correction being used to standardize the amino acid coefficients which may have resulted in an overestimation of digestibility of several amino acids.

Standardized amino acid digestibility of CGM was greater for the PFR and SID than the PFC for most of the amino acids (Table 1). Lys was an exception where there were no differences among assays. The PFR also yielded significantly greater digestibility values for several amino acids when compared to the SID.

When comparing amino acid digestibility values for 3 DDGS samples, the PFR consistently yielded higher digestibility values for DDGS 1 when compared to the PFC (data not shown). The SID also yielded higher values than the PFC for DDGS 1 for most amino acids. The PFR and SID values did not differ except for Met. For DDGS 2, the PFR again yielded significantly higher digestibilities than the PFC for all the amino acids expect Lys. Values for the PFR were also higher than those for the SIAAD for several amino acids. When standardized amino acid digestibilities were determined for DDGS3, values for the PFR and SID were significantly higher than the PFC for several amino acids, but there were no differences among methods for most of the practically important indispensable amino acids.

The standardized amino acid digestibility coefficients of soybean meal and canola meal determined by three different methods are presented in Table 2. For soybean meal, there was very little variation among the methods. The only difference among methods was that Arg and His digestibility in soybean meal was increased in the PFC when compared with the PFR and Asp was significantly increased in the PFR when compared with the SIAAD. For canola meal, the SIAAD yielded more variable results in comparison to the other two methods which were evident by the increased SEM for this assay. The PFR generally yielded greater amino acid digestibilities than the PFC with the SIAAD being intermediate. For Ala, Lys, and His, there were no significant differences among the three methods.

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

Implications

A new precision-fed chick assay (PFC) was developed for determining ileal amino acid digestibility in feed ingredients for chickens. This new assay provides a more rapid and inexpensive method that is complementary to the currently established SIAAD and PFR assays. When the new PFC assay was compared to SIAAD and PFR assays for several feed ingredients, there were no consistent differences in amino acid digestibility among the 3 methods. These results indicate that all 3 assays are acceptable methods for determining amino acid digestibility in feed ingredients for chickens.

Table 1. Comparison of standardized amino acid digestibilities (%) for corn and corn gluten meal determined by three different methods

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

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

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

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

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

Table 2. Comparison of standardized amino acid digestibilities (%) of soybean and canola meals determined by three different methods

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

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

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

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

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