

## **MPRP Annual Report (January 2012)**

### **Further 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. In an earlier study funded by MPRP, we developed and evaluated a new precision-fed ileal chick assay for determining AA digestibility of some feed ingredients. The overall objective of this proposed study was to further evaluate the new precision-fed broiler chick assay for determining ME and AA digestibility of more 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**

A series of experiments were conducted to determine and compare AA digestibility values among the standardized ileal amino acid chick assay, the precision-fed cecectomized rooster assay and the new precision-fed chick ileal assay. The feed ingredients evaluated were 4 corn distillers dried grains with solubles (DDGS) and 4 meat and bone meal (MBM) samples varying in quality.

### ***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 dietary treatments, with 5 birds per pen, 4 replicate pens per experimental diet. The birds were fed the experimental diets for a five day period. On d 21, birds were killed by CO<sub>2</sub> asphyxiation and ileal digesta were collected. The 8 experimental diets were formulated to contain approximately 20% CP with each of the 8 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<sub>2</sub> 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 -20°C until they were processed. For the rooster assay, the excreta were also frozen and stored at -20°C 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 =**

$$[1 - (\text{chromium in diet}/\text{chromium in ileal digesta}) \times (\text{amino acid in digesta}/\text{amino acid in diet})]$$

### **STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY, % =**

$$\text{Apparent digestibility} + [(\text{IEAA flow, g/kg of DMI})/(\text{amino acid content of the diet, g/kg of DM})] \times 100.$$

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, %=**

$$[(\text{Amino acid in feed ingredient (mg)} - \text{Amino acid excreta (mg)} + \text{endogenous amino acid (mg)})/ \text{amino acid in feed ingredient (mg)}] \times 100.$$

## **Results and Discussion**

The results for 3 of the 4 DDGS samples varying in quality (based on color) are presented in Table 1. Digestibility of AA varied among the 3 DDGS samples; however, there were no consistent differences among the 3 assays. For example, the PFR generally yielded higher values than the SIAAD and PFC for DDGS 1 and 2 but not DDGS 3 and differences between the SIAAD and PFC were not consistent. The PFR yielded significantly higher values than the SIAAD and PFC for some AA in DDGS 4 but not for others (data not shown). The last DDGS 4 was very dark in color and had lysine digestibility of only 41-51% for the 3 assays.

For the MBM, 16 samples were evaluated in the PFR and the 2 lowest and 2 highest AA digestibility samples were then evaluated in the PFC. Both assays did a good job of differentiating the poor and high quality MBM which differed greatly in AA digestibility (Table 2). There were differences in AA digestibility between assays, but these were not consistent. The samples were not evaluated in the SIAAD due to insufficient amount of sample.

A few experiments also were conducted to evaluate the new PFC for determining ME of feed ingredients. Unfortunately, it seems that a long excreta collection period of more than 24 hours postfeeding will be needed to collect all dietary residues from material fed. This is likely going to be difficult to get approval from IACUC committees. Alternatively, an ileal digestibility energy value could be obtained from the ileal digesta collected; however, the practical application of these values is questionable. Also, more chicks would also need to be fed to provide enough digesta for bomb calorimeter for energy analyses. Thus, the new PFC seems most useful for AA digestibility determinations.

## **Implications**

A new PFC has been developed for determining ileal AA 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 DDGS and MBM samples, there were no consistent differences in AA digestibility among the 3 methods. These results indicate that all 3 assays are acceptable methods for determining AA digestibility in feed ingredients for chickens.

Table 1. Comparison of standardized amino acid digestibility coefficients (%) for three different corn distillers dried grains with solubles (DDGS) samples determined by three different methods

Item	DDGS 1						DDGS 2						DDGS 3					
	SIAAD <sup>1</sup>	SEM	PFC <sup>2</sup>	SEM	PFR	SEM	SIAAD	SEM	PFC	SEM	PFR	SEM	SIAAD	SEM	PFC	SEM	PFR	SEM
Indispensable																		
AA																		
Arg	77.6 <sup>ab</sup>	1.5	73.4 <sup>b</sup>	3.3	83.2 <sup>a</sup>	0.7	69.3 <sup>b</sup>	1.5	70.6 <sup>b</sup>	1.0	78.8 <sup>a</sup>	0.3	81.5	1.6	80.2	1.7	82.7	1.3
His	74.4 <sup>a</sup>	1.5	64.2 <sup>b</sup>	4.9	78.3 <sup>a</sup>	0.5	62.7 <sup>b</sup>	1.2	60.2 <sup>b</sup>	1.0	67.6 <sup>a</sup>	0.6	80.9 <sup>a</sup>	0.5	74.6 <sup>b</sup>	2.3	78.1 <sup>ab</sup>	0.8
Ile	72.7 <sup>ab</sup>	1.4	66.4 <sup>b</sup>	4.6	78.4 <sup>a</sup>	0.5	58.9 <sup>c</sup>	1.8	65.2 <sup>b</sup>	1.0	73.6 <sup>a</sup>	0.4	78.8	1.2	74.6	2.1	79.1	1.4
Leu	82.1 <sup>ab</sup>	1.4	74.7 <sup>b</sup>	3.8	88.2 <sup>a</sup>	0.4	79.2 <sup>b</sup>	0.8	74.0 <sup>c</sup>	1.2	86.8 <sup>a</sup>	0.2	86.8 <sup>a</sup>	0.5	81.9 <sup>b</sup>	1.7	88.3 <sup>a</sup>	0.9
Lys	61.7 <sup>b</sup>	2.4	58.3 <sup>b</sup>	4.3	69.5 <sup>a</sup>	0.4	37.0 <sup>b</sup>	3.6	57.6 <sup>a</sup>	1.1	57.5 <sup>a</sup>	1.2	65.9	3.1	65.6	2.2	63.5	1.5
Met	78.4 <sup>b</sup>	1.3	76.8 <sup>b</sup>	3.3	86.2 <sup>a</sup>	0.2	69.7 <sup>c</sup>	1.6	74.5 <sup>b</sup>	1.5	83.3 <sup>a</sup>	0.6	84.5	1.3	81.8	1.7	85.2	1.1
Phe	80.9 <sup>a</sup>	1.0	70.7 <sup>b</sup>	4.0	82.1 <sup>a</sup>	0.7	75.4 <sup>a</sup>	1.2	68.8 <sup>b</sup>	1.1	79.4 <sup>a</sup>	0.1	84.8 <sup>a</sup>	1.1	77.8 <sup>b</sup>	1.9	84.6 <sup>a</sup>	1.1
Thr	66.4 <sup>ab</sup>	2.0	55.7 <sup>b</sup>	5.4	70.5 <sup>a</sup>	2.0	61.3 <sup>b</sup>	1.1	56.8 <sup>b</sup>	1.1	67.5 <sup>a</sup>	1.4	73.0	1.2	69.1	2.7	72.6	1.2
Val	71.7 <sup>ab</sup>	1.6	63.5 <sup>b</sup>	4.7	78.5 <sup>a</sup>	0.6	60.6 <sup>b</sup>	1.4	62.2 <sup>b</sup>	0.9	73.5 <sup>a</sup>	1.1	77.6	0.9	73.4	2.4	78.2	1.4
Dispensable																		
AA																		
Ala	80.7 <sup>ab</sup>	1.3	73.7 <sup>b</sup>	3.6	83.4 <sup>a</sup>	0.3	78.5 <sup>a</sup>	0.7	73.3 <sup>b</sup>	1.1	80.9 <sup>a</sup>	0.5	84.6 <sup>a</sup>	0.5	78.7 <sup>b</sup>	1.8	82.9 <sup>a</sup>	1.0
Asp	67.9 <sup>a</sup>	1.6	58.0 <sup>b</sup>	4.7	73.8 <sup>a</sup>	0.9	58.4 <sup>b</sup>	1.2	54.5 <sup>b</sup>	0.7	66.6 <sup>a</sup>	0.8	72.1 <sup>a</sup>	1.2	63.9 <sup>b</sup>	2.6	71.0 <sup>a</sup>	1.6
Cys	72.7 <sup>a</sup>	1.7	53.3 <sup>b</sup>	6.0	77.6 <sup>a</sup>	1.6	62.5 <sup>b</sup>	1.2	49.0 <sup>c</sup>	1.3	68.9 <sup>a</sup>	1.0	80.9 <sup>a</sup>	0.6	65.2 <sup>b</sup>	3.9	75.3 <sup>a</sup>	2.7
Glu	79.1 <sup>ab</sup>	1.5	72.6 <sup>b</sup>	4.0	85.3 <sup>a</sup>	0.5	74.9 <sup>b</sup>	0.8	69.4 <sup>c</sup>	1.1	80.7 <sup>a</sup>	0.1	84.7 <sup>a</sup>	0.6	75.6 <sup>b</sup>	2.5	81.3 <sup>a</sup>	1.2
Pro	80.2 <sup>a</sup>	1.5	68.3 <sup>b</sup>	4.7	84.1 <sup>a</sup>	0.9	75.8 <sup>a</sup>	0.8	68.6 <sup>b</sup>	1.2	79.1 <sup>a</sup>	0.4	85.2 <sup>a</sup>	0.1	75.1 <sup>b</sup>	2.7	82.8 <sup>a</sup>	1.2
Ser	77.1 <sup>a</sup>	1.7	68.0 <sup>b</sup>	4.1	77.4 <sup>a</sup>	0.8	74.6 <sup>a</sup>	0.8	67.2 <sup>b</sup>	0.7	75.5 <sup>a</sup>	2.1	81.5	0.7	76.8	2.2	76.4	2.3
Tyr	81.6 <sup>ab</sup>	1.2	74.7 <sup>b</sup>	3.6	82.4 <sup>a</sup>	0.5	77.4 <sup>a</sup>	1.0	72.2 <sup>b</sup>	1.0	78.7 <sup>a</sup>	0.4	84.9	0.9	81.3	1.8	82.8	1.3

<sup>a,b</sup> Means within a row within DDGS sample with no common superscripts are significantly different ( $P \leq 0.05$ ).

<sup>1</sup>SIAAD=Standardized ileal chick assay; mean of 4 replicate pens of 5 broiler chickens.

<sup>2</sup>PFC=Precision-fed ileal chick assay; mean of 4 replicate pens of 4 broiler chickens.

<sup>3</sup>PFR=Precision-fed cecectomized rooster assay; mean of 4 individual roosters.



Table 2. Digestibility coefficients for some amino acids in meat and bone samples determined in two different assays.

Meat and Bone Meal Sample	Lysine		Cystine		Methionine		Threonine	
	PFR <sup>1</sup>	PFC <sup>1</sup>	PFR	PFC	PFR	PFC	PFR	PFC
Low quality 1	40	35	27	23	53 <sup>a</sup>	36 <sup>b</sup>	46 <sup>a</sup>	31 <sup>b</sup>
Low quality 2	40 <sup>b</sup>	55 <sup>a</sup>	19 <sup>b</sup>	46 <sup>a</sup>	54	56	47	49
High quality 1	81 <sup>b</sup>	87 <sup>a</sup>	67	72	90	89	85	84
High quality 2	78	83	65	66	91 <sup>a</sup>	84 <sup>b</sup>	86 <sup>a</sup>	80 <sup>b</sup>

<sup>a,b</sup>Means within an amino acid and row with different superscripts are different ( $P < .05$ ).

<sup>1</sup>PFR = precision-fed cecectomized rooster assay; PFC = precision-fed ileal chick assay.