# Amino Acid Digestibility: Methods and Practical Use

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### Take Home Message

After total amino acid content, differences in digestibility represent the largest single source of variation in the ability of animals to utilize amino acids from different sources for productive purposes. Failing to take this into account in formulation can result in financial losses, reduced productivity, greater variation in performance, and greater nitrogenous waste production. There are several different methods for estimating amino acid digestibility: each with its own strengths and weaknesses. Formulating diets using SID amino acids offer several advantages over total amino acid formulations including additivity, better prediction of animal performance, better use of lower quality ingredients, reduced nitrogenous waste production and, often, lower feed costs.

Ideally, we would use data generated using animals of the same species and in the same physiological state as those we are feeding. In the absence of ideal data, it is usually better to use less than ideal corrections for digestibility than to use no correction at all.

Digestibility may be altered by differences in processing and storage conditions. Some groups have offered means of estimating heat damage and adjusting digestible amino acids accordingly. Making these adjustments can improve formulation accuracy, but information for making these adjustments must be made rapidly available. NIRS offers a tool that is proving to be useful in providing this type of data when time is of the essence.

### Introduction

High protein ingredients are added to the diet to deliver amino acids to the animal for various metabolic purposes. Although the varied functions of amino acids are all important, financially speaking, our top line is dependent on the amount of high quality animal protein we can produce and sell. We transfer as much of those dollars to the bottom line as we can by focusing on the efficient use of inputs. Since feed is our largest single input cost, it only makes sense to look at feed cost as an opportunity to improve the efficiency of the system.

After energy, protein is the second most expensive component of the diet for most livestock species. Any effort to improve the cost efficiency of livestock and poultry production must, therefore, include a focus on amino acid nutrition.

### Background

In the middle of the last century, protein held a prominent role in feed formulation. Feeds were formulated and marketed on a crude protein basis. This system can be functional when a very limited number of ingredients are used. Since then we have changed our focus to formulations based on amino acids. Protein is now just a vehicle to deliver amino acids to the animal.

In the beginning of the 20$^{th}$ century, scientist knew that proteins were made up of amino acids, but Osborne and Mendel (1914) noted that experiments demonstrating the adequacy of diets containing only completely hydrolyzed proteins to support normally observed growth rates in animals were lacking. Experiments had shown that diets containing only zein and gelatin proteins, known to be

deficient in tryptophan, resulted in little or no growth, and replacing these proteins with "Gliadin" (gluten), known to contain tryptophan, resulted in normal growth. While it was assumed that the growth promoting properties of proteins were due to amino acids, the scientific term of the day for these effective components was "Bausteine" (German for building block). The problem of verifying amino acids as the Bausteine arose from the lack of robust methods for isolating the different amino acids. All proteinogenic amino acids have the same basic structure of an alpha carbon flanked by a carboxyl and an amino group. This structural similarity results in a number of chemical similarities (Vickery and Schmidt, 1931) which complicated attempts at isolation. It was not until 1935 that all of the proteinogenic amino acids could be identified.

A.J.P. Martin and R.L.M. Synge received the 1952 Nobel Prize for developing the technique of partition chromatography to separate amino acids (Martin, 1952). The HPLC-based amino acid analyzers of today are descendants of their work. With automation and other improvements, this technique has allowed us to routinely analyze feed ingredients and complete feeds for their amino acid content. Equipped with this powerful new method, investigators in the 1960's began to catalogue the amino acid content of feed ingredients. Data was available to support formulation on a total amino acid basis. But, feed ingredients vary widely in digestibility and formulating with a variety of protein ingredients requires a common currency. There was a need to move toward bioavailability.

**Methods**

Growth assays are the standard for estimating bioavailability because they demonstrate differences in the ability of different sources to support actual metabolic functions. Growth assays are, however, expensive and produce little data for the effort involved. While digestion and absorption of a nutrient does not guarantee availability for metabolic purposes, these are the major hurdles to be crossed.

The term "digestibility" in the following discussion is used to encompass both digestion and absorption.

The most direct way to measure digestibility is to feed a set amount of feed and collect all of the feces produced. The feces are analyzed for the nutrients of interest and compared to the amount in the feed. The difference is the amount digested. The difference (feed nutrient level – fecal nutrient level) divided by the feed nutrient level is the digestibility coefficient. This method results in Apparent Total Tract Digestibility (ATTD). "Apparent" because it does not take into account amino acid endogenous sources of the nutrient or changes due to fermentation in the hindgut. The Precision Fed Rooster (PFR) Assay is a total tract method originally developed as a TME assay (Sibbald, 1976). Adult roosters are used to avoid complications of growth and to avoid the potential of a laid egg contaminating fecal material. Roosters were fasted for 21 h and then force fed a predetermined amount of the test material via a funnel and tube inserted into the crop. Total feces were collected after 24 h. The method has the advantage that only the test ingredient is fed, there is no to alter the roosters, and the same animals can be used for multiple trials. The method does not account for endogenous losses or changes due to fermentation.

The Precision-Fed Cecectomized Rooster (PFCR) assay is a modification of the PFR assay in which the ceca are surgically removed. The ceca are the main location of fermentation in the gut of poultry (Parsons, 1986). Removing the ceca substantially reduces fermentation induced changes to the amino content of the feces.

The Standardized Ileal Digestibility (SID) chick assay is the *in vivo* method of choice for estimating amino acid availability of feed ingredients. Although the method requires more animals than the precision fed, cecectomized rooster assay, it does offer several advantages over the PFR and PFCR assays (Lemme, 2006). No surgical procedures are necessary. Birds in the assay feed normally as opposed to force feeding. The birds used in the assay can be more similar in age to the birds to be

fed commercially using the assay results. The principle behind the technique is that all amino acid absorption occurs in the small intestine. Any amino acids remaining in the digesta at the terminus of the small intestine may be considered to have missed their opportunity for absorption and, so, are unavailable to the animal. Subtracting these losses from the amino acids ingested gives one estimate of amino acid availability; namely, Apparent Ileal Digestibility (AID).

The thought process is straightforward enough, but several problems have to be worked out. The first issue is how to collect the digesta. In swine this is done by ileal cannulation. A plastic tube with an integrated flange on one end (a so called "T-cannula") is surgically inserted into the ileum just anterior to the ileo-cecal junction. The open end of the T-cannula is exteriorized through the body wall and capped. The cap can be removed for digesta collection, which flows from the cannula under force of normal gut motility or compression of the abdominal cavity by skeletal muscle contraction. This method has been attempted in poultry but proved to be more technically difficult. Poultry researchers have opted for collecting ileal contents by excising the lower ileum post mortem. The contents of the ileal segment are expressed into a container for later analysis.

A second issue for this approach is determining the quantity of feed ingested represented by a given quantity of digesta. In the precision fed rooster assay, the ingredient is given in a known quantity as a bolus and total fecal output is collected for 48 h. In the SID chick assay, birds are fed continuously through an adaptation period of at least days in duration and a single sample is taken at slaughter (Kim, 2010). Relating feed intake to digesta sample size is accomplished by adding an inert, indigestible marker to the feed. As feed ingredients are digested and absorbed, the concentration of the marker in the digesta increases proportionately. The ratio of the concentration of the marker in the digesta to the concentration in the feed is inversely proportional to the ratio of the quantity of digesta to the quantity of feed that is represented by the digesta. Chromic oxide and titanium dioxide are the most commonly used internal markers in poultry digestibility trials.

The third issue to solve is the origin of amino acids in the digesta. We are usually interested in determining the amino acid availability of a single ingredient. While it may be possible to feed some ingredients as the complete diet for a short time, this approach is not suitable for other ingredients due to nutritional inadequacy, palatability or other issues. For this reason, it is standard practice to formulate the experimental diet with the test ingredient as the sole source of amino acids. While this approach settles the issue of dietary amino acid origin, we are still left with endogenous sources of amino acids in the ileal sample.

Mucins, digestive enzymes and sloughed cells from the gut add significant amounts of non-dietary amino acids to the digesta. AID fails to account for these sources can lead to inaccuracies in digestibility estimates (Stein et al, 2007). Correcting AID for endogenous losses gives True Ileal Digestible (TID) values. TID has a clear advantage over AID, since it more accurately accounts for the availability of amino acids of dietary origin only. There is another, less immediately obvious advantage. AID values for some feed ingredients are not additive when combined in a feed formula (Stein et al, 2005). When we formulate feeds we generally make the assumption that nutrients from different sources are additive. That is, if 100 kg of ingredient A contains 5 units of a nutrient and 100 kg of ingredient B has 3 units of the same nutrient, then we would expect that 200 kg of a mixture of equal parts A and B would contain 8 units of the nutrient. This is not the case for AID amino acids in some ingredients. With low dietary amino acid concentrations, endogenous losses make up a larger portion of ileal amino acid content than they do with high dietary amino concentrations.

These endogenous losses can be partitioned into Basal losses and Specific losses. Basal losses are largely the cost of maintaining a healthy, functional gut and are well correlated to dry matter intake. Physiological state and experimental conditions (like surgery) can affect basal losses. Specific endogenous losses are those losses which are affected by characteristics of the feed ingredients

such as content and the presence of antinutritional factors and other factors that affect mucin production, enterocyte turnover rate, etc.

Basal endogenous losses can be estimated several different ways. Most commonly, researchers feed a nitrogen-free diet to the same or similar birds. Any amino acids collected in the digesta under these conditions is of endogenous origin. Collection of ileal contents from fasting animals has also been used to determine endogenous losses (Parsons, 2002). Fasting animals do not have the same stimulation to the gut tract as fed animals and are in a different physiological state than fed animals (Low, 1990).

There are currently no methods for directly measuring specific losses, but there are indirect methods. There is little data available for TID due to the difficulty and expense of accurately determining these specific endogenous losses. As a result, TID is not currently recommended for feed formulation.

Correcting AID for basal endogenous losses alone produces values for Standardized Ileal Digestibility (SID) amino acids. SID values are additive and, so, are useful in formulation.

**Practical Use**

SID amino acid values provide a means of using different ingredients or different qualities of the same ingredients to obtain the same performance. While it would be optimal to have digestibility values for each load of ingredients, this is not currently practical. The most reliable solution at the moment is to analyze for total amino acids and calculate digestible amino acid content using average digestibility coefficients.

The table on the right shows the value of using SID amino acid values when using alternative protein sources.

Diet 1 is a corn/soybean meal broiler diet formulated to the Evonik SID Lysine recommendation. All other minimum essential amino acid levels were set as a ratio to Lys according to the ideal protein concept. Diet 2 is formulated to the same total amino acid level with canola meal and meat and bone meal. In this case SID Lys is below the recommendation and probably more limiting than Met or TSAA. Performance will likely be below that obtainable from Diet 1. Diet 3 uses the same ingredients as Diet 2, but is formulated to the same SID Lys level as Diet 1 and would be expected

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Corn | 55.4 | 50.7 | 49.7 | 52.4 |
| Soybean Meal 48 | 35.9 | 21.6 | 22.2 | 19.9 |
| Corn Oil | 5.2 | 6.6 | 6.7 | 6.3 |
| Canola Meal | | 15 | 15 | 15 |
| Meat & Bone Meal | | 5 | 5 | 5 |
| Dical | 1.58 | | | |
| CaCO3 | 0.87 | 0.5 | 0.44 | 0.45 |
| Salt | 0.31 | 0.26 | 0.26 | 0.26 |
| DLM | 0.25 | 0.2 | 0.2 | 0.19 |
| Premix | 0.2 | 0.2 | 0.2 | 0.2 |
| Biolys | 0.1 | 0.18 | 0.23 | 0.25 |
| Na Carb | 0.04 | | | |
| L-Thr | 0.01 | | 0.03 | 0.03 |
| L-ILE | | | 0.02 | 0.02 |
| ME, MJ/kg | 13.2 | 13.2 | 13.2 | 13.2 |
| **Total Amino Acids** | | | | |
| Lys | 1.23 | 1.23 | 1.28 | 1.23 |
| Met | 0.56 | 0.56 | 0.56 | 0.54 |
| Met+Cys | 0.92 | 0.95 | 0.95 | 0.91 |
| Thr | 0.84 | 0.84 | 0.88 | 0.85 |
| Trp | 0.26 | 0.26 | 0.26 | 0.24 |
| **Digestible Amino Acids** | | | | |
| Lys | 1.11 | 1.07 | 1.11 | 1.07 |
| Met | 0.53 | 0.52 | 0.52 | 0.49 |
| Met+Cys | 0.82 | 0.82 | 0.82 | 0.79 |
| Thr | 0.71 | 0.67 | 0.71 | 0.68 |
| Trp | 0.23 | 0.22 | 0.21 | 0.2 |
| Crude Protein | 21.4 | 22 | 22.4 | 21.5 |
| Cost per Ton | 452.67 | 443.84 | 440.84 | 438.37 |

to perform equally to Diet 1. Diet 4 has the same ingredients and SID Lys level as Diet 2, but the ratios of the other amino acids have been corrected to reflect the ideal protein profile. Performance of Diet 4 should be similar to 2, but with lower cost and a lower crude protein, resulting in less nitrogenous waste.

One advancement on this approach is the ability to adjust digestibility coefficients for heat damage. Heating is the most common processing method for feed ingredients. It is used for drying, solvent recovery, and destruction anti-nutritional factors. Too much heat can destroy amino acids and cause Maillard reactions which are particularly damaging to Lysine. For almost two decades, Evonik has been using NIRS technology for estimating amino acid content in a range of feed ingredients. Some heat damaged materials were producing amino acid estimates that were not as expected. In the process of adjusting for this problem, the analytics group found a way to reliably predict heat damage in a sample (heat damage index; HID) and then equate this damage to a change in SID values. This technology is also NIRS based allowing rapid, low cost predictions for SID amino acids in several ingredients of plant origin and has been dubbed AminoRED, for Rapid Evaluation of Digestibility.

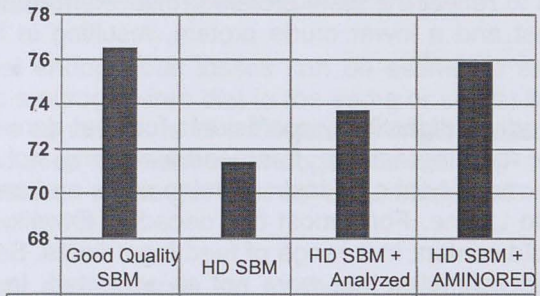
AminoRED has been validated for soy products and corn based DDGS for broilers. The following graphs represent data from the soy products validation carried out at the University of Wageningen, the Netherlands.

Ross 308 broilers (960 birds, 10 d of age) were allocated to one of four dietary treatments. In treatment 1, the diet was formulated with good quality soybean meal. The soybean meal for the other treatments was heat damaged by heating for 120 min at > 115 C and 9 bars. For treatment 2, the heat damaged meal was formulated into the diet using the same nutrient loadings as those for the good quality meal. For treatment 3, the heat damaged meal was analyzed for total amino acids and the diet was formulated using those values and standard digestibility coefficients. For treatment 4, the SID values of the heat damaged meal were adjusted using AminoRED®.

Treatment 2 is analogous to the situation of receiving a poor quality ingredient without recognizing it as such; a very realistic situation resulting in depressed performance. If we are careful enough to analyze for total amino acids and adjust our nutrient matrix and, perhaps, supplementation of amino acids, we can recover a portion of the expected performance as in treatment 3. Finally, adjusting for the effects of heat damage allows us to recover to virtually 100% of the performance we could achieve with good quality soybean meal.

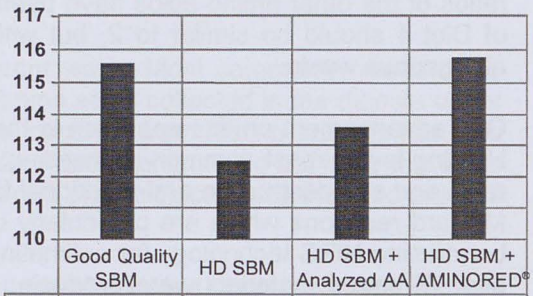
Formulating diets using SID amino acids offer several advantages over total amino acid formulations including better prediction of animal performance, better use of lower quality ingredients, reduced nitrogenous waste production and, often, lower feed costs.

**Daily bodyweight gain, g/d**




| | Good Quality SBM | HD SBM | HD SBM + Analyzed | HD SBM + AMINORED |
|---|---|---|---|---|
| BW | 76.5 | 71.4 | 73. | 75.9 |
| <0.05 | a | c | b | a |

**Feed intake, g/d**

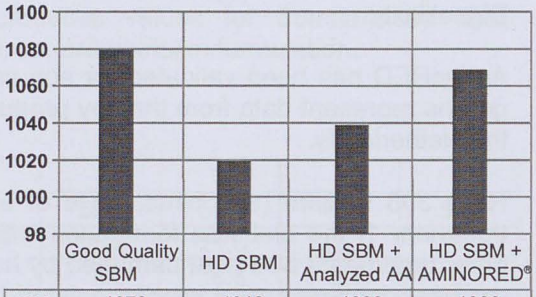



| | Good Quality SBM | HD SBM | HD SBM + Analyzed AA | HD SBM + AMINORED® |
|---|---|---|---|---|
| FC | 116 | 113 | 114 | 116 |
| <0.05 | a | b | b | a |

**Feed conversion ratio, feed/unit gain**


| | Good SB | HD SBM | HD SBM Analyzed AA | HD SBM + AMINORED |
|---|---|---|---|---|
| FCR | 1.51 | 1.58 | 1.54 | 1.53 |
| <0.05 | a | c | b | ab |

**Carcass weight, g**




| | Good Quality SBM | HD SBM | HD SBM + Analyzed AA | HD SBM + AMINORED® |
|---|---|---|---|---|
| CW | 1079 | 1019 | 1039 | 1069 |
| <0.05 | a | c | b | a |

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## NOTES