- Invited Review -
Evaluation of Amino Acid and Energy Utilization in Feedstuff for Swine and Poultry Diets

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ABSTRACT: An accurate feed formulation is essential for optimizing feed efficiency and minimizing feed cost for swine and poultry production. Because energy and amino acid (AA) account for the major cost of swine and poultry diets, a precise determination of the availability of energy and AA in feedstuffs is essential for accurate diet formulations. Therefore, the methodology for determining the availability of energy and AA should be carefully selected. The total collection and index methods are 2 major procedures for estimating the availability of energy and AA in feedstuffs for swine and poultry diets. The total collection method is based on the laborious production of quantitative records of feed intake and output, whereas the index method can avoid the laborious work, but greatly relies on accurate chemical analysis of index compound. The direct method, in which the test feedstuff in a diet is the sole source of the component of interest, is widely used to determine the digestibility of nutritional components in feedstuffs. In some cases, however, it may be necessary to formulate a basal diet and a test diet in which a portion of the basal diet is replaced by the feed ingredient to be tested because of poor palatability and low level of the interested component in the test ingredients. For the digestibility of AA, due to the confounding effect on AA composition of protein in feces by microorganisms in the hind gut, ileal digestibility rather than fecal digestibility has been preferred as the reliable method for estimating AA digestibility. Depending on the contribution of ileal endogenous AA losses in the ileal digestibility calculation, ileal digestibility estimates can be expressed as apparent, standardized, and true ileal digestibility, and are usually determined using the ileal cannulation method for pigs and the slaughter method for poultry. Among these digestibility estimates, the standardized ileal AA digestibility that corrects apparent ileal digestibility for basal endogenous AA losses, provides appropriate information for the formulation of swine and poultry diets. The total quantity of energy in feedstuffs can be partitioned into different components including gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy based on the consideration of sequential energy losses during digestion and metabolism from GE in feeds. For swine, the total collection method is suggested for determining DE and ME in feedstuffs whereas for poultry the classical ME assay and the precision-fed method are applicable. Further investigation for the utilization of ME may be conducted by measuring either heat production or energy retention using indirect calorimetry or comparative slaughter method, respectively. This review provides information on the methodology used to determine accurate estimates of AA and energy availability for formulating swine and poultry diets. (**Key Words:** Chickens, Digestibility, Methodology, Pigs)

INTRODUCTION

For efficient production of animal products, the right amount of nutritionally adequate feedstuff should be supplied to the animal. All dietary components, such as energy, amino acid (AA), vitamins, and minerals are important when formulating diets for swine and poultry, however more attention should be given to the dietary energy and AA, as these components account for major cost of swine and poultry diets. A deficiency of essential AA and energy results in a reduction in performance. In addition, the excess of these components in the animal diets above the requirements is excreted and consequently could be a detrimental source to the environment. Therefore, it is important to formulate diets that meet energy and AA requirements for swine and poultry while at the same time minimizing the excretion of excess energy and N into the

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environment. To achieve this goal, the digestion characteristics and utilization of feedstuff should be well understood. The objective of this review is to focus on appropriate methodology that is used to determine the accurate estimates of AA and energy utilization for the formulation of swine and poultry diets.

METHODOLOGICAL CONSIDERATIONS ON DIGESTIBILITY STUDY FOR SWINE AND POULTRY

Quantitative feed and feces (total collection) method

A standard digestibility study, in which the digestibility of a component in test feedstuff is determined, requires measuring the ingested amount of that component and the voided amount of given component of test feedstuff. The total collection and index methods have been widely used to determine the digestibility of components in swine and poultry diets. The total collection method requires an accurate measure of feed intake and fecal output for determining the amount of the component ingested and voided via feces, respectively. With these measurements, the digestibility of the component can be calculated as follows:

$$\text{Digestibility}\,(\%) = \left[\frac{C_{input} - C_{output}}{C_{input}}\right] \times 100$$

where C_{input} and C_{output} are the amount of component ingested and voided via the feces, respectively.

In a total collection study for swine, pigs are individually housed in crates and thereafter they are adapted to their crates and feed being given before fecal sample collection. The adaptation period usually lasts for 3 to 7 days before a collection period of 4 to 6 days (Adeola, 2001). During the adaptation period, a feeding level is adjusted to avoid feed refusal which results in additional work such as orts collection during the collection period as well as drying and analyzing the orts after the collection period. A level of feeding at 3 times maintenance (197 kcal/kg BW$^{0.60}$; NRC, 2012) or approximately 3% to 4% of body weight (BW) per d is suggested as the sufficient level of feeding for the digestibility study with total collection method. During the collection period, colored markers such as ferric oxide, chromic oxide, and indigo carmine are commonly used for the identification of fecal output from a given ingested feed (Adeola, 2001; Kim et al., 2006; Son et al., 2013). Once pigs are adjusted to the crates and feed, the collection period begins and ends with feeding the first and the last marked feed, respectively. In this period, the feces that voided between the first and second appearances of the marker are collected as the representative output that is associated with the fed quantities given in the collection period.

In a balance study, it is difficult to identify urine that belongs to specific feed because marker does not appear in the urine, thus the urine collection is generally conducted based on time. The quantitative urine collection starts from the day of the first marked feed offered and ends at the day of the last marked feed. With measurement of components in the urine, the metabolizability of the component can be calculated as follows:

$$\text{Metabolizability}\,(\%) = \left[\frac{C_{input} - C_{output} - C_{urine}}{C_{input}}\right] \times 100$$

where C_{urine} is the amount of component voided via the urine.

In a digestibility study for poultry, the total collection method is not common for determining the fecal digestibility, because feces and urine are voided together in the form of excreta and it is difficult to separate the feces from the excreta and measure digestibility. There was an attempt to avoid this confounding effect of urine on the fecal digestibility using surgical technique such as colostomy (Okumura, 1976), however there are problems with the artificial anus including skin regrowth, intestinal stasis and hardening of fecal material (Paulson, 1969). Thus, the total collection method in poultry usually involves collecting excreta (feces+urine). Sibbald (1976) developed the precision-fed rooster assay and McNab and Blair (1988) later suggested some modification. In this assay, adult cockerels or roosters were fasted for 48 h prior to being fed test ingredients. During the fasting period, all birds are tube-fed 2 doses of 25 to 30 g of glucose (as an aqueous solution) at 8 and 32 h post-feed withdrawal, which partly alleviates the effects of starvation. At 48 h post-feed withdrawal, all birds are tube-fed 25 to 30 g of their assigned test ingredients that are in distilled water and ground through a 0.5 mm screen prior to feeding. Birds for determining endogenous losses are fed 50 g of glucose. The total collection of excreta is conducted for 48 h after feeding of test ingredients or glucose for endogenous losses determination. During 48-h collection period, all birds are given 50 ml of water by tube about 32 h after feeding to overcome any effects induced by low water intake.

Index method

The total collection method involves laborious quantitative records of feed intake and output whereas the index method can avoid these laborious procedures, but greatly relies on accurate chemical analysis of index compound in the feed and fecal output. In the use of an index, there are inherent fundamental assumptions which include that index compound should be i) completely inert

in the gastrointestinal tract, ii) completely and regularly excreted, and iii) uniformly mixed with the digesta or fecal material. Thus, the amount of index compound in the feed and the amount voided in the output should be uniform over equal periods of time (Adeola, 2001). Several index compounds including chromic oxide, titanium dioxide and insoluble ash are commonly used for the determination of digestibility (Jagger et al., 1992; Betancourt et al., 2012; Kim et al., 2012; Olukosi et al., 2012) and are added to the diet at 0.1% to 0.5%. With the index method, digestibility is calculated as follows:

$$\text{Digestibility (\%)} = 100 - \left[\left(\frac{CI_{input} \times CC_{output}}{CI_{output} \times CC_{input}}\right) \times 100\right]$$

where CI_{input} and CI_{output} are the concentration of index compound in feed and feces, respectively; CC_{input} and CC_{output} are the concentration of component in feed and feces, respectively.

Direct and difference (indirect) methods

The digestibility of components in test ingredient is determined either by the direct or by the difference (indirect) method. In the direct method, the test diet is formulated such that all the component of interest is supplied by the test ingredient alone. This method is simple and only one test diet is required for determining digestibility of components in the test ingredient. In some cases, however, a test ingredient cannot be fed for a long enough period of time due to low palatability and anti-nutritional factors. Also, it may not be possible to supply all the component of interest with the test ingredient alone. Therefore, it may be necessary to formulate basal diet and a test diet in which a portion of the basal diet has been replaced by the test ingredient. The fundamental assumption of this method is that there is no interaction between the digestibility values of components in the test ingredient and the basal diet. With any of the direct or the indirect method, the digestibility of the component can be determined using total collection or index method and is calculated as follows (Adeola, 2001; Adeola and Kong, 2014):

$$D_{td} \times P_{td} = (D_{bd} \times P_{bd}) + (D_{ti} \times P_{ti}) \qquad \text{Eq (1)}$$

in which D_{bd}, D_{td}, and D_{ti} are the digestibility (%) of the component in the basal diet, test diets, and test ingredient, respectively, and P_{bd} and P_{ti} are the proportional contribution of the component by the basal diet and test ingredient to the test diet, respectively. By definition,

$$P_{td} = P_{bd} + P_{ti} = 1 \text{ or } P_{bd} = 1 - P_{ti} \qquad \text{Eq (2)}$$

Solving Eq (1) for D_{ti} gives

$$D_{ti} = \frac{D_{td} - (D_{bd} \times P_{bd})}{P_{ti}}$$

Substituting Eq (2) in Eq (1) gives

$$D_{ti} = \frac{D_{td} - [D_{bd} \times (1 - P_{ti})]}{P_{ti}} = D_{bd} + \frac{D_{td} - D_{bd}}{P_{ti}}$$

A regression method can be used to determine the digestibility of components by having serial proportions of the test ingredient replacing the basal diet. The regression of the digestibility of the component against proportions of the component replaced and extrapolation to 100% replacement is used to determine digestibility of components in test ingredient (Adeola, 2001).

EVALUATION OF AMINO ACID DIGESTIBILITY IN FEEDSTUFF

Relative bioavailability of amino acids

The AA composition of a feed ingredient can be determined by chemical analysis. This however does not provide information on the amount of AA in ingredient that is available to the animal. This information is rather obtained through a well-designed relative AA bioavailability or AA digestibility study.

The slope-ratio assay has been used to determine the relative AA bioavailability that provides relative information on the capacity of feedstuff to supply a specific limiting nutrient and to promote growth (Lewis and Bayley, 1995; Adeola, 1996; Kim et al., 2006). In this assay, a basal diet that is deficient in a specific AA of interest, is supplemented with graded levels of either crystalline form or test AA source of interest and thereafter a dose-response relationship between either crystalline form (standard response relationship) or test ingredient and response criteria is established. Comparison between relationship is then conducted (Batterham, 1992). There are 3 assumptions for validity of the slope-ratio assay and procedures to determine whether a particular slope-ratio assay is valid, should be conducted (Littell et al., 1997). These test procedures are made sequentially and include the test for linearity of the slopes and lack of curvature; the test for equality of intercepts; and the test for intersection of responses to standard (crystalline or synthetic) and test diets at the zero level (blanks).

Because animal growth represents all of the components that can affect bioavailability (digestion, absorption, and utilization), the growth assay is usually considered as the absolute standard for estimating bioavailable AA against

other methods (Ravindran and Bryden, 1999). However, this assay is time-consuming, expensive, and limited in that only one AA can be assessed at a time. Moreover, this has relatively high standard error and gives relative values only (Parsons, 2002). Thus, the slope-ratio method is not practical and applicable for all AA in all feed ingredients and diets for the animal.

Ileal digestibility of amino acids

Due to the limitation of the slope-ratio assays, there has been a need for the development of assays that are more rapid and practical for determining AA availability. Digestibility assays have been widely used for estimating the availability of AA in feed ingredients. The digestibility of AA represents a portion of total dietary AA that is enzymatically hydrolyzed, fermented by microbes in the digestive tract and absorbed from the digestive tract (Fuller, 2003). Depending on the site of sample collection, the digestibility estimates of AA can be divided into two measurements. The fecal or excreta digestibility of AA determines the difference between the amounts of AA ingested and excreted in the feces of pigs or excreta of birds, respectively. However, because the digested AA and small peptides are primarily absorbed from the small intestine and the unabsorbed AA is altered by microbial fermentation in the hindgut, there is a question whether digestibility values measured by comparing dietary intakes and fecal or excreta outputs gives credible estimates of AA availability (Zebrowska, 1973; Just et al., 1981; Mosenthin et al., 1992). For poultry species, because ceca are the main site of microbial fermentation and cecectomized birds whose ceca are surgically removed were used to avoid the microbial alteration of AA (Parsons, 1986) and a large volume of excreta AA digestibility data were generated using the precision-feeding assay with cecectomized cockerels (Parsons, 2002). However, the results of AA digestibility studies between cecectomized and intact birds were inconsistent and may vary with the type of feed ingredients (Green et al., 1987; Ragland et al., 1999). Moreover this method suffers from the major concern which is the application of digestibility values generated in adult cockerels to growing birds because physiologically mature birds may not reflect the digestive capability of younger growing birds (Garcia et al., 2007). For swine, there is an evident study for the superiority of ileal over fecal digestibility of AA in practical diet formulation for growing pigs (Dierick et al., 1988). In this study, the results indicated that there was a higher correlation between weight gain and ileal nitrogen digestibility (r = 0.76) or feed conversion and ileal nitrogen digestibility (r = –0.87) than fecal nitrogen digestibility (r = 0.34 and –0.65, respectively).

Due to the aforementioned reasons, the ileal digestibility rather than fecal or excreta digestibility of AA in feed ingredients is considered to be more accurate estimate of AA digestibility. The ileal digestibility of AA is determined prior to microbial degradation and synthesis in the hindgut and avoids confounding effect of urine on the AA digestibility (Ravindran and Bryden, 1999). In addition, for poultry study, the test diets can be fed *ad libitum* as well as birds of various ages can be used in the ileal digestibility assays, which allows examination of differences in digestibility between ages under physiologically normal feeding conditions.

Ileal digesta collection methods

Ileal digesta can be collected either directly from the ileum after euthanasia (slaughter method) or through an intestinal cannula. For swine, the most preferred ileal digesta collection method is the simple T-cannula insertion method because this is the least invasive method and does not involve the surgical resection of parts of the lower digestive tract (Stein et al., 2007). In this method, a simple T-cannula is surgically inserted 10 to 15 cm anterior to the ileocecal junction (Sauer and de Lange, 1992) and ileal digesta samples are collected through the cannula after the pigs have been on the experimental diets for 5 to 10 d. The pigs are usually fed twice daily and samples are collected over an 8- to 12-h period following feeding. An indigestible index compound is used to calculate the digestibility of AA. It is also possible to collect fecal samples prior to collecting the ileal digesta. Cannulated pigs can be repeatedly used to determine the ileal digestibility of a number of diets with Latin square or randomized design. However, the cannula method has not been commonly used for poultry digestibility study because of the difficulties in the surgical as well as in the collection procedures such as sample blockage and cannula rejection (Parsons, 2002). The most widely used method for the ileal digestibility estimation in poultry is the slaughter method. In this method, birds are fed an experimental diet containing index compound for several days (4 to 5 d) and thereafter the birds are euthanized and digesta samples are collected from the terminal ileum section which is defined as the section of the gastrointestinal tract extending from the Meckel's diverticulum to a point 20 mm cranial to the ileocecal junction (Rezvani et al., 2008). Although the majority of AA is absorbed prior to ileum, the distal half or two third of ileum is the preferred site for ileal digesta collection to ensure marker recovery and complete absorption of AA (Kadim and Moughan, 1997; Rezvani et al., 2008). Pooling of collected ileal digesta from several birds (8 to 10 birds) in the same cage is also involved to obtain sufficient ileal digesta sample for analyses. Because of the nature of ileal collection, ileal contents cannot be collected quantitatively,

thus the use of indigestible index compound is added to the test diets.

Apparent ileal digestibility of amino acid

The terminology describing the ileal digestibility of AA in feed ingredients has been well defined in the previous review (Stein et al., 2007). In this review, depending on the contribution of ileal endogenous AA losses in AA digestibility calculation, the ileal digestibility of AA can be expressed as apparent ileal digestibility (AID), standardized ileal digestibility (SID), or true ileal digestibility (TID). The AID of AA is defined as the net disappearance of ingested dietary AA from the digestive tract proximal to the distal ileum and can be calculated as follows:

$$AID(\%) = 100 - \left[\left(\frac{C_{input} \times AA_{output}}{C_{output} \times AA_{input}} \right) \times 100 \right]$$

where C_{input} and C_{output} are the concentrations (g/kg) of index compounds in feed and ileal digesta dry matter (DM), respectively; AA_{input} and AA_{output} are the concentrations (g/kg) of AA in feed and ileal digesta DM, respectively.

By this definition, the apparent ileal AA digestibility inherently does not differentiate between dietary and endogenous origin AA in the outflow at the distal ileum. Therefore, the level of AA in the test diet affects the AID of AA (Eklund et al., 2008). With low-protein diet, the relative contribution of endogenous AA to total AA in the ileal outflow is high and as the crude protein level in the diet increases, the relative contribution decreases, therefore a primary concern with the use of AID in diet formulation is lack of additivity when mixed diet contains the low-protein ingredients (Kong and Adeola, 2013a).

Ileal endogenous amino acid losses

Amino acids in the outflow at the terminal ileum contain AA from dietary origin as well as various endogenous proteins such as digestive secretions (saliva, bile, gastric, and pancreatic secretions as well as intestinal secretion), mucoproteins, sloughed intestinal epithelial cells, serum albumin, and amide (Moughan and Schutter, 1991; Ravindran and Bryden, 1999). Those of endogenous proteins at the terminal ileum constitute the ileal endogenous AA losses that are divided into the basal and specific losses (Jansman et al., 2002; Stein et al., 2007). The basal endogenous AA losses are related to the dry matter intake (DMI) but independent of the type of feedstuff or diet. In contrast, the specific AA losses are related to the composition of the feedstuff or diet and therefore induced by specific feed ingredient characteristics such as contents and types of fiber, anti-nutritional factors, and level of dietary protein.

Several methods have been used to determine the basal endogenous AA losses including the feeding a highly-digestible protein, regression method, and feeding an N-free diet. Feeding a highly-digestible protein method includes casein or hydrolyzed casein at a very low level in the diet (usually between 4% and 7% of the diet) and it is assumed that AA in the diet may be completely digested and absorbed. Thus, all the AA in the digesta collected are assumed to be of basal endogenous losses. The most common method for determining basal endogenous AA losses is the feeding an N-free diet method. Even though there are several considerations including an overestimation of endogenous losses of Pro and Gly, underestimation of overall AA, and non-physiological approach compared with feeding a highly-digestible protein method, the feeding an N-free diet method may be preferred over the other methods because of its simplicity in methodology. In addition, estimates of basal endogenous AA losses derived from pigs and broilers fed an N-free diet are comparable to those from the feeding a highly-digestible protein or the regression method (Jansman et al., 2002; Adedokun et al., 2007). When N-free diet containing an index compound is used, the basal endogenous losses of AA are calculated as follows:

$$Basal\,endogenous\,loss\,(g/kg\,of\,DMI)$$
$$= \left(\frac{C_{input}}{C_{output}} \times AA_{output} \right)$$

The values of the basal endogenous AA losses are affected by feeding levels and BW (Park et al., 2013). In addition, the ingredient composition of the N-free diet such as dietary fiber, index compound and major carbohydrate contents (Adedokun et al., 2011; Kong and Adeola, 2013b; Kong et al., 2014) may affect the basal endogenous AA losses and thus standard N-free diets (Table 1) were suggested to minimize variation derived from the use of different ingredient composition of N-free diets across experiments (Stein et al., 2007; Adedokun et al., 2011).

The specific AA losses are estimated by calculating the difference between the total (specific plus basal) and basal losses of AA (Lemme et al., 2004). The procedures, including the homoarginine technique, the feeding enzyme-hydrolyzed protein and the isotope-dilution technique, are used for estimating the total endogenous losses of AA (Hodgkinson et al., 2003). However, these methods are laborious, expensive, and require specialized equipment and as a consequence, the estimates for total endogenous losses are not routinely determined for feed ingredient evaluation (Stein et al., 2007).

True and standardized ileal digestibility of amino acids

The TID or SID of AA can be calculated by correcting

Table 1. Suggested ingredient composition of the standard N-free diets from the literature (g/kg, as-fed basis)

| Ingredient | Pigs[1] | | Broilers[2] |
	Nursery	Growing-finishing	
Corn starch	545	791	200.5
Dextrose	150	100	640
Lactose	200	-	-
Vegetable oil	30	30	50
Synthetic fiber	30	40	50
Limestone	5	5	13
Monocalcium phosphate	24	19	19
Indigestible index	4	4	5
Vitamin-mineral premix[3]	2	2	5
Potassium carbonate	4	4	2.6
Magnesium oxide	1	1	2
Salt	5	4	-
Sodium bicarbonate	-	-	7.5
Choline chloride	-	-	2.5
Potassium chloride	-	-	2.9
Total	1,000	1,000	1,000

[1] Stein et al. (2007).
[2] Adedokun et al. (2011).
[3] Vitamins and minerals in the premix should be adequate for the requirement of pigs (NRC, 2012) and broilers (NRC, 1994).

AID values for total or basal endogenous AA losses, respectively. Thus, the TID is the relationship between only undigested dietary AA (not the endogenous AA losses) in the ileal outflow and dietary AA intake whereas the SID of AA can reflect TID as well as feed ingredient effects on the specific endogenous AA losses. In addition, SID values are more likely to be additive in mixed diet for swine and poultry compared with AID values (Stein et al., 2005; Kong and Adeola, 2013a). Therefore, the SID rather than the AID and TID of AA is considered to be more appropriate for the formulation of swine and poultry diets and are calculated as follows:

$$SID\ (\%) = AID + \left[\left(\frac{\text{Basal endogenous loss}}{AA_{input}} \right) \times 100 \right]$$

EVALUATION OF ENERGY DIGESTIBILITY AND UTILIZATION IN FEEDSTUFF

The utilization of energy in feedstuffs for pigs may be determined by total collection method in which pigs are fed test diet over a period of time and feces and urine are collected for subsequent chemical analysis. For poultry, the classical apparent metabolizable energy (ME) method, in which feed intake and excreta out are recorded for a 2- to 5-day test period or the ratio of DM intake to output is determined by index compound, was used to determine energy utilization (Hill and Anderson, 1958; Sibbald and Slinger, 1962). The precision-fed method (Sibbald, 1976; McNab and Blair, 1988) described above has also been widely used for determining the ME in feedstuffs. Depending on the collected energy-containing components (feces and urine), either the digestible energy (DE) or ME can be determined for pigs whereas due to the difficulty in methodology, only the ME is commonly determined for poultry. The DE and ME can be determined either the direct or indirect (difference) method (Table 2) depending on feed ingredient to be tested and calculated as follows (Adeola, 2001):

$$DE\ (\text{kcal/kg DM}) = (GE_I - GE_F)/DMI$$

$$ME\ (\text{kcal/kg DM}) = (GE_I - GE_F - GE_U)/DMI$$

where GE_I, GE_F, and GE_U are GE intake, output in feces, and output in urine (kcal/d), respectively; DMI is dry matter

Table 2. Example calculation of energy digestibility by the difference method[1]

	Basal diet (BD)		Test diet (TD)		Test ingredient (TI)	
Energy yielding component in diets (g/kg)		967.5		967.5		1,000
TI (g/kg)		0		300		1,000
Gross energy (as-is basis, kcal/g)		3.927		4.103		4.985
Dry matter (DM, %)		88.41		88.76		92.74
Gross energy (DM basis, kcal/g)	3.927÷88.41×100	4.4418	4.103÷88.76×100	4.6226	4.985÷92.74×100	5.375
Energy from BD (DM basis, kcal/kg)	4.4418×1,000	4,441.8	3.927×(967.5−300) ÷967.5×1,000÷88.41×100	3,064.5		
Energy from TI (DM basis, kcal/kg)			4.985×300÷92.74×100	1,612.6		
Energy from BD+TI (DM basis, kcal/kg)			3,064.5+1,612.6	4,677.1		
Proportional contribution of energy by TI to TD (P_{ti})			1,612.6÷4,677.1	0.3448		
Digestibility of energy in BD (D_{bd}, %)[2]		86.22				
Digestibility of energy in TD (D_{td}, %)[2]				80.40		
Digestibility of energy in TI (D_{ti}, %)					86.22+(80.40−86.22) ÷0.3448	69.34

[1] Data were taken from Adeola and Kong (2014).
[2] Determined by either the total collection or index method.

intake (kg/d).

The endogenous losses of energy, including digestive enzymes, sloughed-off cells, and intestinal microbial activity products may be determined by fasting birds and collecting excreta in the precision-fed method. Correcting the apparent ME for the endogenous losses provides the true ME and these values are always greater than or equal to apparent values and are not affected by the dietary energy level whereas apparent values are affected (Adeola, 2001). With using the precision-fed method described above, the true ME can be calculated as follows:

$$TME \text{ (kcal/kg DM)} = ME + EEL/FI$$

where TME is true ME content of feed; EEL is the endogenous energy loss (kcal) from the feed-deprived birds; FI is the intake of the test feedstuffs (kg).

Because the energy that is deposited as retained protein in mature animals cannot be completely recovered by animals if the AA are degraded for energy, both apparent and true ME can be corrected to N equilibrium with using the correction factor of 7.45 or 8.22 kcal/g of N for pigs or birds, respectively (Hill and Anderson, 1958; Harris et al., 1972). But this correction to N equilibrium may not be valid for growing pigs that retain considerable amounts of N which is not usually used as an energy source (NRC, 1998; Kil et al., 2013). The N-corrected ME is calculated as follows:

$$ME_n \text{ (kcal/kg)} = ME - (F_C \times NR)$$

where ME_n is N-corrected ME; F_C is the correction factor of 7.45 or 8.22 kcal/g of N for pigs or birds, respectively; NR is N retention, g/kg DMI.

Metabolizable energy is used primarily for the basal metabolism which is the minimum activity required for sustaining animal's life including cellular activity, respiration, circulation, muscular activity, secretion and excretion. If there is an additional energy intake in excess of the requirement for the basal metabolism, then the energy is retained in the body (Lizardo et al., 2002). Therefore, the measurement of either the energy retained in the animal's tissue or the animal's total heat production is required to determine the utilization of ME. The heat production, which is the difference between ME intake and energy retention, is often measured by indirect calorimetry in which oxygen consumption and production of carbon dioxide and methane as well as urinary N are measured for calculation of heat production using the equation proposed by Brouwer (1965):

Heat production (kcal)
$$= (3.866 \times O_2) + (1.2 \times CO_2) - (0.518 \times CH_4) - (1.431 \times N_u)$$

where O_2 represents the litters of oxygen consumed, CO_2 and CH_4 represent the litters of carbon dioxide and methane produced, respectively, and N_u represents the grams of urinary N produced. The energy retained may be measured by the comparative slaughter technique in which a representative group of animal is slaughtered at the beginning of a period and another representative group is slaughtered at the end of the period and the difference between the energy at the beginning and end of the period is the energy retained.

CONCLUSION

In a digestibility study for swine and poultry, methodology for determining digestibility estimates should be carefully selected. For the AA digestibility, ileal rather than fecal digestibility should be considered to provide meaningful estimates. It is also suggested that apparent ileal AA digestibility be corrected for endogenous AA losses especially low-protein feedstuff is evaluated with the direct method. Standardized ileal AA digestibility that is corrected for basal endogenous AA losses provides more accurate information for the formulation of animal diets. In the energy utilization studies, the total collection method is suggested for determining DE and ME in feed ingredient for pigs whereas for poultry the classical metabolizable energy assay and the precision-fed method are applicable. Further investigation for utilization of ME may be conducted by measuring either heat production or energy retention using the indirect calorimetry or the comparative slaughter method, respectively.

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