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Evaluation of Collection Method and Diet Effects on Apparent Digestibility and Energy Values of Swine Diets

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EVALUATION OF COLLECTION METHOD AND DIET EFFECTS ON APPARENT DIGESTIBILITY AND ENERGY VALUES OF SWINE DIETS

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Two experiments were conducted to investigate the effects of collection method and diet on estimating digestibility values. In Exp. 1, 24 barrows were fed either a corn-soybean meal diet (CSBM) or CSBM with 20% dried distillers grains with solubles (DDGS). Fecal collections were based on exact timing (Day collection) or marker-to-marker collection (MM) methods during a 4-d period. Diets contained 0.5% of titanium dioxide (TiO_2) for estimating digestibility using the index method. In Exp. 2, the effects of collection method and basal diet on determination of DDGS digestibility were studied using 24 barrows. Diets were CSBM (basal 1), barley-canola meal diet (BCM; basal 2), and 20% of basal 1 or basal 2 replaced by DDGS (total 1 and total 2). Day and MM methods were administered for each individual pig by separate collections and measurements of feces. In Exp. 1, Day and MM methods were not different ($P > 0.10$) on estimation of digestibility values, except that ME values tended to be greater ($0.05 < P < 0.10$) when estimated using Day vs. MM methods; whereas, digestibility estimates and dietary energy values were about 0.5% and 20 kcal/kg lower ($P < 0.05$) estimated using the index vs. total collection (Day and MM) methods. In Exp. 2, digestibility estimates of diets and DDGS were not different ($P > 0.10$) calculated using Day and MM methods.

The average DE and ME (kcal/kg, as-fed) of DDGS were 4,035 and 3,704, respectively estimated using basal 1, which were not different (*P* > 0.10) from using basal 2 (4,081 and 3,651, respectively). In conclusion, digestibility values of a complex diet and DDGS are not different when estimated by Day or MM method. When corn-soybean meal based diets are fed, digestibility estimates are lower using the index method compared to the total collection method. Additionally, basal diets may not affect DDGS digestibility estimates.

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DEDICATION

May all the glory be to the Lord God.

To my father Li Shu-Huai and my mother Fan Ying.

In all these things we are more than conquerors through him who loved us. For I am convinced that neither death nor life, neither angels nor demons, neither the present nor the future, nor any powers, neither height nor depth, nor anything else in all creation, will be able to separate us from the love of God that is in Christ Jesus our lord. (Romans 8:37-39)

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Chapter 1: Literature Review

INTRODUCTION

To optimize feed efficiency and minimize feed cost, it is very important to determine nutrient availability and dietary energy content of feed ingredients. Apparent total tract digestibility (ATTD) values are usually expressed for digestibility of DM, CP, P, gross energy (GE), lipids, and dietary fiber (DF; NRC, 2012). Disaccharides and starch are normally assumed to be 100% digestible (van Beers et al., 1995; Stein and Bohlke, 2007). The AA concentration in feces is considered to not accurately represent the amount of unabsorbed AA in the small intestine due to *de novo* synthesis from microbial fermentation in the large intestine (Sauer and Ozimek, 1986) and the contribution of endogenous AA in feces (Nyachoti et al., 1997). Therefore, the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) values are expressed for AA digestibility values (Stein el al., 2007). The basal endogenous AA losses are considered by the SID values, and these values are believed to be additive in mixed diets (Stein et al., 2005). The procedures for conducting digestion and balance studies were discussed by Adeola (2001), Gabert et al. (2001) and Stein et al. (2007). The total collection (TC) and the index method are the 2 major techniques for estimating ATTD of nutrient and energy in vivo. For total collection of feces, the time-based (Day) and marker-to-marker (MM) collection methods are commonly used.

The partitioning of energy in the pig was defined by Ewan (2001). Gross energy (GE) is the quantity of heat resulting from the complete combustion of feed or other substances, which can be measured using an adiabatic bomb calorimeter. Digestible energy (DE, kcal/kg) can be achieved by quantitatively subtracting the amount of energy

excreted in the feces from GE intake; and metabolizable energy (ME, kcal/kg) is subsequently achieved by subtracting the amount of energy excreted in urine and gas from DE. The ME represents 92 to 98% of DE in pigs (NRC, 2012). The net energy (NE) system considers heat increment (HI) in ME and more accurately expresses the utilization of energy by dividing it into NE for maintenance (NE_m) and for production. The sum of HI and NE_m equals total heat production (HP). Because the estimate of NE is relatively costly, DE and ME systems are normally used for non-ruminants instead of NE system. Metabolism crates have been used for quantitatively collecting feces and urine in pigs for measuring DE and ME; and the collection of gas is usually omitted, because gas losses is only 0.5% DE for conventional diets fed to growing-finishing pigs (Noblet et al., 1994).

The objective of this chapter is to review the factors affecting nutrient and energy digestibility in pigs, and to compare the different methodologies used for estimating ATTD values.

FACTORS AFFECTING NUTRIENT DIGESTIBILITY OF PIGS

Dietary factors

*CP concentration***.**

Dietary CP concentration positively affects the apparent CP digestibility of pigs. Kerr and Easter (1995) reported that reducing dietary CP from 16% to 12% decreased apparent CP digestibility of growing pigs. This is due to the decreased endogenous N losses with increasing dietary CP concentrations (Fan et al., 1995). Greater dietary CP concentration did not affect fecal N losses, but increased urinary N losses (Quiniou et al.,

1995). In contrast, Le Bellego et al. (2001) reported that both fecal and urine N excretions were increased with increasing dietary CP concentrations; and apparent CP digestibility of high-CP diet was greater than that of low-CP diet.

AA pattern.

Balanced dietary AA pattern improved apparent CP digestibility in a low-CP diet due to the decreased fecal N excretion (Kerr and Easter, 1995), and subsequently achieved similar growth responses as pigs receiving a high-CP diet (Kerr et al., 2003). These results indicated the possibility of greater digestibility of crystalline AA in comparison to protein-bound AA or a negative effect of crystalline AA on endogenous and exogenous losses of N in the gastrointestinal tract (GIT).

Dietary fiber.

Dietary fiber is defined as the carbohydrates that are resistant to digestion by mammalian enzymes (AACC, 2001). Dietary fiber decreases apparent nutrient digestibility by increasing endogenous and exogenous (i.e., dietary) losses, or decreasing the hydroxylation and absorption of dietary protein and peptides, or both. Endogenous sources of N in the large intestines include secretions from the circulation, sloughed cells and microbiota (Fuller and Reeds, 1998; Bergen and Wu, 2009). Schulze et al. (1994) reported that the inclusion of increasing amount of purified neutral detergent fiber (pNDF) in the diet increased ileal exogenous N loss, and subsequently decreased AID of DM, CP, and ash. In addition, the increased total N flow by wheat bran diet vs. a pNDF diet included 35% of endogenous origin (0.499 g of N/kg of DMI) and 65% of exogenous origin (0.907 g of N/kg of DMI; Schulze et al., 1995). Using the ^{15}N isotope dilution

technique, Leterme et al. (2000) reported that, the total ileal endogenous N loss increased with the increasing levels of barley (20.7% total DF). Pigs fed high-fiber diets had increased fecal DM excretion and decreased ATTD of CP (Wilfart et al., 2007). Dietary fiber may boost the passage rate, diminish the effectiveness of hindgut fermentation, and decrease the *de novo* synthesis and reabsorption of AA in the large bowl (Fuller and Reeds, 1998); subsequently, it decreases the apparent fecal digestibility values.

Animal factors

Several studies have reported that breed and age/body weight affect nutrient and energy digestibility of pigs.

Breed.

Due to the larger intestines relative to their BW, Meishan pigs were shown to have greater ATTD of DM, GE, and some nutrients in corn-soybean meals and in DDGS than Yorkshire pigs at an identical age or similar body weight (Urriola et al., 2012). Comparatively, modern crossbred pigs have the capacity of using soluble dietary fiber (SDF) as effectively as old local breeds (von Heimendahl et al., 2010), indicating that the breed effect on nutrient digestibility may be associated with the DF sources used.

Age.

According to Le Goff and Noblet (2001) and Jørgensen et al. (2007), the ATTD of GE and fiber were not affected by age if large proportions of SDF diets are fed. Nevertheless, when pigs were fed high insoluble dietary fiber (IDF) diets, sows had the greatest ATTD of GE and fiber, followed by finishing pigs, compared to growing pigs. It was concluded that differences in the size of intestines and differences in the capacity of

microbes to ferment IDF may contribute to the greater apparent digestibility values by older pigs than by younger pigs. Noblet and van Milgen (2004) suggested that the DE values of some ingredients were greater for adult sows vs. growing pigs.

Endogenous secretion decreased with increased BW (Fuller and Reeds, 1998). Hess and Sève (1999) reported that pigs at lower BW (45 kg) had greater endogenous losses of CP and AA than those at greater BW (77 kg). Mariscal-Landín et al. (2008) found greater specific activity of chymotrypsin and carboxypeptidase A in grower pigs than in piglets, which resulted in greater ileal CP and AA digestibility capacity for heavier pigs. Moreover, there were linearly decreased basal endogenous losses relative to DM intake in pigs at the lower BW; whereas, the values were constant for heavier pigs regardless of the feeding level (Hess and Sève, 1999). It was suggested that the AA digestibility is not affected by age, BW, and physiological condition if the BW of the pig is greater than 60 kg (Stein and Nyachoti, 2003). Similarly, the ATTD of phosphorus in pigs at 60, 75, and 90 kg of BW were not different (Kemme et al., 1997).

Feed intake

The effect of feed intake (FI) on apparent digestibility values is affected depending on the site of the GIT and the nutrient composition of the diet. Haydon et al. (1984) reported that increasing FI tended to improve the AID of nutrients, but also decreased the ingested nutrients that disappeared in the large intestine, which subsequently decreased ATTD of DM and numerically decreased ATTD of CP. At lower FI, the longer retention time (Seerley et al., 1962) and less non-protein substrate for microbes’ requirement may result in increased deamination of AA by microbes and

absorption of the large intestine (Fuller and Reeds, 1998). At the ileum level, endogenous losses of CP and AA (IAA_{end}) expressed as gram per day increased with the increasing FI (Hess and Sève, 1999; Moter and Stein, 2004). When IAA_{end} was expressed as g/kg DMI, basal IAA_{end} linearly decreased from feeding level at 50 to 90 g DM/kg $BW^{.75}$(Hess and Sève, 1999). In addition, it was suggested that the basal IAA_{end} (g/kg DMI) should be measured at feeding levels greater than 70 g DM/kg $BW^{.75}$ due to the relatively constant values between the medium and high feeding levels (Hess and Sève, 1999). Moter and Stein (2004) reported quadratic increases in the AID of CP and the means of AID of indispensable and dispensable AA as FI increased from 30 to 90 g DM/kg $BW^{.75}$; whereas, the basal IAA_{end} (g/kg DMI) linearly decreased. After correcting AID for IAA_{end}, standard ileal digestibility coefficients (SID) of CP and the means of indispensable and dispensable AA decreased linearly as FI increased (Moter and Stein, 2004). Interestingly, Goerke et al. (2012) reported that the AID and SID of CP and AA increased at FI between 30 and 45 g/kg BW and decreased between 45 and 60 g/kg BW as FI increased.

Conflicting results have been published on the effect of FI on apparent energy digestibility of pigs. No difference was observed for the ATTD of GE at different FI levels (Moter and Stein, 2004). In contrast, Haydon et al. (1984) reported decreased energy digestibility with increasing FI. Harris et al. (2012) selected pigs based on their residual feed intake (RFI), and concluded that the digestibility values for DM, CP, and GE were greater in the low vs. high RFI pigs; whereas, P digestibility did not differ between the pig lines.

FACTORS AFFECTING ENERGY UTILIZATION IN PIGS

Protein concentration and profile

Increasing dietary CP level increases dietary energy values but decreases the efficiency of DE convert to ME. Additionally, diet with balanced amino acid profiles benefits energy utilization. Energy digestibility was not affected by CP concentration, but the ME:DE ratio was lower for high-CP diet than for low-CP diet due to the greater urinary energy losses (Quiniou et al., 1995; Atakora et al., 2011). A similar tendency on NE:ME ratio related to dietary CP concentrations was also shown (Noblet et al., 1993). A reduction in DE was observed in diets with reduced CP concentration, but ME was not affected (Le Bellego et al., 2001). Comparatively, Moehn et al. (2013) did not find differences in DE between low- and high-CP diet; whereas, ME for low-CP diet were greater than high-CP diet regardless of FI level. With adequate AA supplementation, reduction of dietary CP did not affect energy gain and heat production in piglets (Le Bellego and Noblet, 2002). However, improved energy utilization, particularly due to lower HP and greater lipid deposition, in growing pigs fed low-CP diet has been shown (Bellego et al., 2001). These results indicate that the effect of supplementation of AA in low-CP diet on energy utilization may depend on BW. Similarly, Kerr et al. (2003) reported lighter kidney weight and less HP in pigs fed low-CP diet supplemented with AA; and pigs on low- and high-CP diet achieved similar growth performance.

Fiber

In general, DF negatively affects apparent digestibility coefficient of energy, because digestibility of DF is much lower than for other nutrients and DF dilutes the

energy concentration of the diet (Noblet et al., 1993; Galassi et al., 2010). The average ATTD of total dietary fiber (TDF) in 24 sources of DDGS was only 47.3% (Urriola et al., 2010). Digestibility of TDF varies among different DDGS sources (Urriola et al., 2010), which may result in different ATTD of GE in the 14 DDGS sources (Pedersen et al., 2007; Stein et al., 2009). Fermentation of DF primarily produces volatile fatty acid (VFA; acetic, propionic, and butyric acids), gas (CO_2, CH_4, and H_2), heat, urea, and bacterial mass, which decrease the efficiency of energy utilization from digestible fiber (Noblet and Goff, 2001). Dietary fiber increased energy losses as CH_4 and the HP as a proportion of ME, and consequently, decreased the retained energy in growing pigs (Jørgensen et al., 1996) and in adult sows (Ramonet et al., 2000). Contrary to these studies, others reported that DF did not affect HP and energy retention (Schrama et al., 1996; Goff et al., 2002). These contrasting results may due to the difference of variation in DF levels between diets and the different housing systems (Goff et al., 2002).

Dietary fiber has a less pronounced negative effect on digestibility of energy in sows vs. growing pigs; and this difference is dependent on the DF source (Le Goff and Noblet, 2001). On high-fiber diet, sows have better efficiency of utilization of energy derived from hindgut fermentation than growing pigs (Jørgensen et al., 2001). Soluble dietary fiber is more digestible than IDF, because it is much more fermentable (Urriola et al., 2010). Sows fed high concentrations of SDF diet had less activity than those fed high level of IDF diet (Serena et al., 2008), which was likely due to the greater water-binding capacity and viscosity of soluble fiber that delayed the gastric emptying (Miquel et al., 2001).

Fat

Dietary fat contributes positively to the efficiency of energy utilization. Increasing dietary lipids increases NE of diets (Kil, 2008). The improvements due to balanced AA profiles on energy digestibility and utilization were accentuated when fat was added to a low-CP diet (Noblet et al., 2001). The NE:ME ratio was greater in diets containing greater fat concentrations (Noblet et al., 1993). In addition, the apparent digestibility of intact fat (63.2%) was lower than that of extracted fat (81.9%; Kil et al., 2010), indicating that the form of dietary fat may also affect dietary ME and NE values.

METHODOLOGIES OF ESTIMATING NUTRIENT AND ENERGY DIGESTIBILITY

Quantitative feed and feces (Total collection) method

The fundamental assumption of total collection (TC) method is that after a sufficiently long adaptation period with constant FI level and frequency, the digestion and metabolism processes reach homeostasis in vivo. The amounts of feces and urine can be estimated for individual pigs housed in the metabolism crates during a collection period, in which FI is also recorded. Feed, feces and urine samples can be analyzed for nutrient and energy concentrations, which are then used to calculate nutrient and energy inputs and outputs. Digestibility and metabolizability of the nutrients and energy can be calculated as follows:

$$Digestibility, \% = 100 \times \left(\frac{Component_{intake} - Component_{feces}}{Component_{intake}}\right)$$

(Eq. 1-1)

$$Metabolizability, \% = 100 \times \left(\frac{Component_{intake} - Component_{feces} - Component_{urine}}{Component_{intake}}\right)$$

(Eq. 1-2)

where $Component_{intake}$, $Component_{feces}$, and $Component_{urine}$ represent the amount of component consumed, voided in the feces, and voided in the urine, respectively.

(Adeola, 2001)

Feces collection.

The total collection of feces can be determined via 2 methods: one is the time-based collection (Day) method, and the other is the marker-to-marker collection (MM) method. Using the Day method, fecal collection is initiated from the beginning of the record of FI and is ceased immediately prior to the next meal after FI recording period (Liu et al., 2012). The feces that are collected during the Day method period are not all belonging to the FI that is recorded. Thus, errors in fecal collection may cause under or over-estimation of digestibility values. However, if daily FI is timed, limited and constant during the adaptation and collection period, daily fecal output should remain relatively constant. It is important to monitor daily FI over the early adaptation period and adapt all animals to a constant FI during the following days before the collection period.

For the MM method, feces belonging to a given feed are determined by feeding a colored and indigestible compound (marker) at the first and the last meals, respectively during the FI recording period. The commonly used markers are ferric oxide, indigo carmen, chromic oxide, etc. (Adedokun and Adeola, 2005; Stein et al., 2011; Kim et al.,

2009). The first and second appearances of the colored feces represent the beginning and the end of the fecal collection, respectively. The quantity of feces collected is taken to represent the fecal output from the first marked meal to the meal before the second marked meal (Adeola, 2001). This method also assumes that the marker has the similar transit rate in the GIT with the digesta and it does not diffuse into the adjacent unmarked digesta. Nevertheless, the assumption may be incorrect and the palatability of feed may be affected by the marker (Lammers et al., 2008). Moreover, the separation of colored and uncolored feces can be somewhat subjective (Liu et al., 2012). Although the use of a marker may improve the precision of fecal collection, the same procedure is not adopted in urine collection because the marker does not appear in urine (Pedersen et al., 2007).

Day collection vs. marker-to-marker collection.

To date, limited data have been reported that directly compare the digestibility estimates or dietary energy values using the Day vs. MM methods. Lammers et al. (2008) conducted 5 experiments using barrows at different BW fed increased levels of crude glycerol. In Exp. 1 and 2, the MM method was used, but they found the pigs seemed to have an aversion to the diets containing marker (ferric oxide). Therefore in Exp. 3 through 5, the Day method was used with the same adaptation and collection period in 2 groups of nursery pigs and 1 group of growing pigs. The apparent DE and ME of crude glycerol were not affected by the different collection methods. Energy digestibility and metabolizability averaged approximately 90% and 86%, respectively for the 5 experimental diets with the addition of 10% crude glycerol, indicating that the digestibility estimates were similar using the different collection methods (Lammers et

al., 2008). Using the Day method, Liu et al. (2012) reported that the energy content of different particle sizes of DDGS ranged from 3,738 to 4,006 kcal of DE/kg of DM and 3,583 to 3,862 kcal of ME/kg of DM, respectively. Similar ME values (3,575 to 3,976 kcal/kg) were observed in 4 sources of DDGS estimated using the MM method; whereas, the DE values (3,922 to 4,252 kcal/kg) were greater than Liu et al.'s data (Stein et al., 2009). In addition, the DE and ME of corn-based diet using the Day method were 3,883 and 3,805 kcal/kg of DM, respectively (Anderson et al, 2012), which are similar with estimates using the MM method (3,921 and 3,825 kcal/kg of DM, respectively; Pedersen et al., 2007).

Urine collection.

For a balance study, urine is collected over preservatives to limit bacterial growth and to trap ammonia (Liu et al., 2012). The commonly used preservatives include hydrochloric acid, sulfuric acid, and formaldehyde (Kim et al., 2012; Pedersen et al., 2007; Adeola, 2001). Currently, urine belonging to a given feed is not able to be determined because markers do not appear in the urine. Thus, urine collection is time-based. Most of the metabolism experiments collected urine during the same period as fecal collection using the Day method (Anderson et al., 2012) or during the first and last marked meals in the MM method (Stein et al., 2011). Comparatively, Kim et al. (2009) fed pigs the marker in the morning meals of d 8 and d 13, but they conducted urine collection from 1700 h on d 8 to 1700 h on d 13 instead. In another study, urine collection was initiated 14 h after the first marked meal and ceased 14 h after the second marked meal (Agudelo et al., 2007). There are few studies that have compared the nutrient and

energy balance estimates using the different urine collection procedures. Additionally, no study has been published to investigate the urine collection period in accordance with the marker-based fecal collection period.

Index method

The alternative method to test the apparent digestibility of nutrient and energy is the index method, which avoids the complete collection of feed and feces using the metabolism crates. Implementing this method, a daily diet with a certain concentration of the index compound (marker) is consumed by the animals. It is assumed that the similar amount of index compound as consumed is transited through the GIT and voided in the feces. Feed and fecal samples are collected and analyzed for concentrations of nutrient, energy and the index compound; the digestibility of nutrient and energy can be calculated as follows:

$$Digestibility, \% = \left(1 - \frac{[index]_{feed} \times [component]_{feces}}{[index]_{feces} \times [component]_{feed}}\right) \times 100$$

(Eq. 1-3)

where $[index]_{feed}$ and $[index]_{feces}$ represent concentrations of index compound in feed and feces, respectively; $[component]_{feed}$ and $[component]_{feces}$ represent concentrations of a test component in feed and feces, respectively.

(Adeola, 2001)

The index method is also commonly used for evaluating ileal digestibility estimates.

Markers used in the index method should have the following properties: chemically analyzable, totally indigestible and unabsorbable, nontoxic, pass through the

GIT in a constant rate, and homogeneously mixed in the feed and feces (Jagger et al., 1992; Adeola, 2001). Chromic oxide (Cr_2O_3), acid insoluble ash (AIA), and titanium dioxide (TiO_2) are commonly used index compounds to determine ileal and fecal apparent digestibility values (Jagger et al., 1992; Jørgensen et al., 1997; Scott and Boldaji, 1997). The inclusion concentration of the index compound in the diet is usually between 0.1 to 1.0%. The accuracy of apparent digestibility estimates using the index method is correlated with the recovery of an index compound, which is the quantity recovered from feces excretion expressed as a proportion of that consumed (Kavanagh et al., 2001).

Chromic oxide vs. titanium dioxide.

Examination of marker excretion patterns in ewes showed that the mean concentrations of TiO_2 excreted in fecal samples were consistently greater than those of Cr_2O_3 across all diet types (an all forage diet, a 50% forage diet, and a 75% concentrate diet; Myers et al., 2006). In pigs, Jagger et al. (1992) reported that lignin (98.1%) and TiO_2 (96.9%) at 0.5% inclusion level had better recovery rates than Cr_2O_3 (79.7%) at 0.5% inclusion level, which resulted in lower ATTD of CP and AA calculated using Cr_2O_3 vs. TiO_2 and lignin. Moreover, the lowest standard errors were obtained with TiO_2 and the greatest with Cr_2O_3 at 0.1% inclusion level. Therefore, TiO_2 was suggested to be the most suitable index compound in studies utilizing pigs at the concentration of 1 g/kg diet (Jagger et al., 1992). It should be noted that feed was not measured in this study; thus, digestibility estimates might be underestimated using the index method. Moreover, the average ileal recovery of TiO_2 (95 to 100%) was greater than that of Cr_2O_3 (83 to

87%), resulting in low estimates of ileal digestibility using Cr_2O_3, while the TiO_2 marker showed good agreement with values estimated using the TC method (Yin et al., 2000). In contrast, Kavanagh et al. (2001) reported that the recovery rate for Cr_2O_3 (96.0%) was greater than for TiO_2 (92.3%), indicating that the recovery of the respective index compound varies among studies.

Chromic oxide vs. acid insoluble ash.

Van Leeuwen et al. (1996) suggested that Cr_2O_3 may be more suitable than acid insoluble ash (AIA) for estimating digestibility in pigs, even though the Cr_2O_3 content in the ileal digesta for wheat gluten meal was not as constant as for soybean meal. In their experiment, different digestibility estimates were estimated between Cr_2O_3 and AIA among different diets, but they were not consistently higher or lower for either marker. Comparison between the index and the TC method showed that Cr_2O_3 was superior to AIA for estimating apparent fecal digestibility in pigs (Bakker and Jongbloed, 1994), which may due to the weaker acid (3*N*) used in this study (Kavanagh et al., 2001). However, adverse results were reported in broiler chickens. Acid insoluble ash was more sensitive than Cr_2O_3 for detecting the expected differences in apparent metabolizable energy (AME) between barley-based diets with and without enzymes (Scott and Boldaji, 1997).

Index vs. total collection method.

Normally, fecal samples from a consecutive collection period are pooled and subsampled for comparison of the index method (IM) and TC. With the same nutrient composition results in the feed and feces, the major difference between the two methods

is derived from the quantity of index compound recovered from feces excretion (the recovery rate). Lower digestibility values should be observed using the index vs. the TC method, when marker recovery is below 100%. The ATTD and AID of DM, OM, ash, CP, and ether extract evaluated with the index method (Cr_2O_3) were consistently lower than values estimated using the TC method in cannulated pigs (Mroz et al., 1996). The ATTD of GE and DM estimated using Cr_2O_3 and AIA were similar with using the TC method; whereas, estimates using TiO_2 were lower than the TC method due to the poor fecal recovery of TiO_2 (Kavanagh et al., 2001). In dogs, digestibility coefficients estimated by Cr_2O_3 and by TC were similar; and differences were lower than 1% (Carciofi et al., 2007).

Agudelo et al. (2010) conducted digestibility experiments in pigs using the IM (Cr_2O_3) immediately after the experiment previously assessing TC (Agudelo et al., 2007). Single grab sampling decreased the accuracy of digestibility estimates by IM (especially for mineral digestibility values) compared to the multiday sampling procedure. The IM method seemed not a suitable method for estimating digestibility of micronutrients compared to the TC method. It was also suggested that at least a 5-d adaptation period is necessary for the index compound content to be stabilized in feces (Agudelo et al., 2010).

Diurnal variations of marker in the feces.

A study using 2 pigs fed different amounts and frequencies of meals showed that the excretion patterns of Cr_2O_3 and CP, and Cr_2O_3 and ash corresponded closely (correlation coefficients were 0.9846 and 0.9837, respectively); whereas, the inverse relationship was observed between Cr_2O_3 and crude fiber (CF) contents in the feces

(correlation coefficient was -0.8856). The single dose of Cr_2O_3 that was administered in the morning meal (6 h) took a shorter time to appear in the feces than when it was administered in the afternoon meal (17 h). Dissection and analysis of the GIT contents 4 h after ingested Cr_2O_3 indicated that CF travels through the GIT at a much slower rate than the bulk of the dry matter; whereas, Cr_2O_3 was removed at about the same rate as DM (Moore, 1957).

Pond et al. (1986) conducted an experiment, in which the partial ingredients of the diets were either marked with Cr mordant or rare earths to monitor the passage rate of feed residues and particle markers. Separate fecal samples were collected every 6 h for 7 d after the marked diets were fed. The residence time due to displacement flow, or in other words the first marker appearance, were 30.0, 25.8, and 25.5 h, respectively in the basal diet (corn-soybean meal), alfalfa diet (basal with 20% alfalfa meal), and corn cobs diet (basal with 10% corn cobs). Those values for dietary components (corn, alfalfa meal, and SBM) had the similar patterns in the respective diets. Corn and SBM had shorter residue time values in the corn cobs diet than in the basal diet (approximately 26 h vs. 30 h). These results indicated that the greater fiber content in the alfalfa and corn cobs diets shortened the passage rate of feed residues and particle markers. Jørgensen et al. (1997) also suggested that less digestible components of a diet have shorter transit times.

Direct and difference (indirect) methods for estimating digestibility of ingredients

Digestibility of components in a test feedstuff can be determined by direct or the difference method. In the direct method, the diet is formulated primarily with the test feedstuff so that all the components of interest are contributed by the test feedstuff.

Normally, the direct method can be used for determining digestibility of ingredients with high feeding value (e.g., cereal grains).

In other cases, the test feedstuff is not able to be used as the major ingredient to supply all the interested components or has poor palatability. The diets are therefore formulated with both the test feedstuff and other feedstuffs, which also supply the interested components, known as the difference (indirect) method. This method assumes that there are no interactions between the digestibility values of components in the test feedstuff and the basal diet. The determination of digestibility of feedstuff using direct or difference method was described by Adeola (2001).

The regression method is also used for estimating feedstuff digestibility. One group of pigs are fed the basal diet and another groups of pigs are fed diets with at least 2 proportions of the basal diet that are replaced by the test feedstuff (Fan and Sauer, 1995).

Comparison of direct, difference, and regression method.

Digestibility values of CP and AA in barley and canola meal were determined using direct, difference, and regression methods (Figure 1.1; Fan and Sauer, 1995). Using the difference method, AID of CP and AA in barley were increased with increasing contributions of CP and AA in barley to the total dietary contents; whereas, the adjusted digestibility values of CP and AA in canola meal were similar among different inclusion levels. The different digestibility patterns in barley and canola meal may be due to the relatively greater contribution of CP from canola meal vs. barley to the corresponding diets (57.4 to 85.8% vs. 14.2 to 42.6%). In addition, decreased standard errors were observed with increased inclusions of the test feedstuff (barley or canola meal). For the

regression method, linear relationships and the corresponding digestibility values in barley and canola could not be obtained for some of the AAs, indicating larger differences of AA digestibility among the feedstuffs were required for determining AA digestibility with the regression method.

Comparing digestibility values determined with direct, difference (data from the greatest inclusions of the test ingredients, barley and canola meal, were used), and regression method suggested that the direct method was not suitable for determining AID of CP and AA in low-protein feedstuffs, such as barley; and either direct or difference method can be used in high-protein feedstuffs, such as canola meal. A relatively high dietary inclusion concentration of test feedstuff was suggested for the difference method to achieve more reliable measurements. Also, the regression method should be used for feedstuffs with poor palatability (Fan and Sauer, 1995).

The DE and ME of wheat were not different using direct (3,953 and 3,889 kcal/kg DM) and regression method (3,960 and 3,876 kcal/kg DM; Bolarinwa and Adeola, 2012). Using the regression method, the true total-tract digestibility of phosphorus (TDP) in the mixture of corn and SBM (37.52%) was not different from the expected value (37.92%) calculated using the TDP of corn (40.53%) and SBM (35.96%), indicating that the regression method is a reliable technique to derive TDP of feedstuffs (Zhai and Adeola, 2013). However, the TDP of SBM estimated using the regression method varied among studies (48.5 to 50.7%, Fan et al., 2001; 51.3%, Ajakaiye et al., 2003; 45.2%, Dilger and Adeola, 2006; 35.96%, Zhai and Adeola, 2013).

Comparison of using different basal diets.

Using the difference or regression method, various basal diets have been used to determine digestibility of nutrients in a test ingredient. May and Bell (1971) conducted an experiment in which different CP concentrations of basal diets were used to determine digestibility of fishmeal at inclusion levels of 25% and 50% and wheat at 50%. Only numerical differences were observed in digestibility of GE and CP for both ingredients using different basal diets, as well as DE and ME of wheat. When a low-CP basal diet was used, DE and ME of fishmeal at inclusion level of 25% were 9.2% and 12.4%, respectively greater than those estimated at 50%; whereas, when high-CP basal diet was used, DE and ME values were 9.1% and 10.3% lower, respectively at the inclusion level of 25% vs. 50%. These results suggest that difference of CP contributions to the total diet between basal diet and the test feedstuff may affect digestibility estimates of the ingredient.

Stein et al. (2005) suggested that using a low-protein ingredient (e.g., cereal grains) resulted in underestimation of predicted AID of CP and the majority of AA in corn-soybean meal and corn-canola meal based diets compared to the measured values. The differences were mainly due to the greater endogenous AA losses observed in the low-protein diet (Fan et al., 1995). Similar differences between the directly determined and predicted apparent P digestibility values have also been published (Fan and Sauer, 2002). It was suggested that the digestibility coefficients based on AID are not as additive as values based on SID when low-CP ingredients are included in the mixed diet (Stein et

al., 2005). Thus, it is possible that the apparent digestibility values of the same ingredient are different using different basal diets.

To compare the effect of different basal diets on the determination of apparent digestibility, apparent DE and ME values of feedstuffs, 12 studies are listed (Table 1.1). The differences among studies for the same test ingredients were probably due to different basal diets (especially due to different CP concentrations), the various sources of ingredients, or the different experimental conditions.

SUMMARY

There is increasing interest in the determination of nutrient digestibility and energy content of feed ingredients. This literature review discussed the factors (i.e., dietary, animal, and FI) that may affect the apparent digestibility and energy values of diets. Comparisons among studies have shown no major differences between the Day vs. MM methods on apparent energy digestibility and dietary energy values. Digestibility estimates determined using the index method were either similar or lower than those using the TC method (Kavanagh et al., 2001). The latter more precisely estimated the apparent digestibility values for minerals (Agudelo et al., 2010). The diurnal variations of marker in the feces were also discussed.

For estimating digestibility of a specific ingredient, the direct method is generally used for evaluating high-quality feedstuffs; using the difference method, more reliable measurements can be achieved when a relatively high dietary contribution of the test ingredient is mixed in the assay diet; also, the regression method is suitable for evaluating the ingredient with poor palatability (Fan and Sauer, 1995). Different CP concentrations

in the basal diet affected the apparent digestibility and energy values of the test feedstuff (May and Bell (1971), but the effect of completely different basal diets on evaluating digestibility of feedstuff has not been investigated. Therefore, the main focus of this research is to compare nutrient digestibility and dietary energy values of diets and ingredients determined using the Day and the MM method, and additionally, to discuss the effect of different basal diets on digestibility estimates of DDGS.

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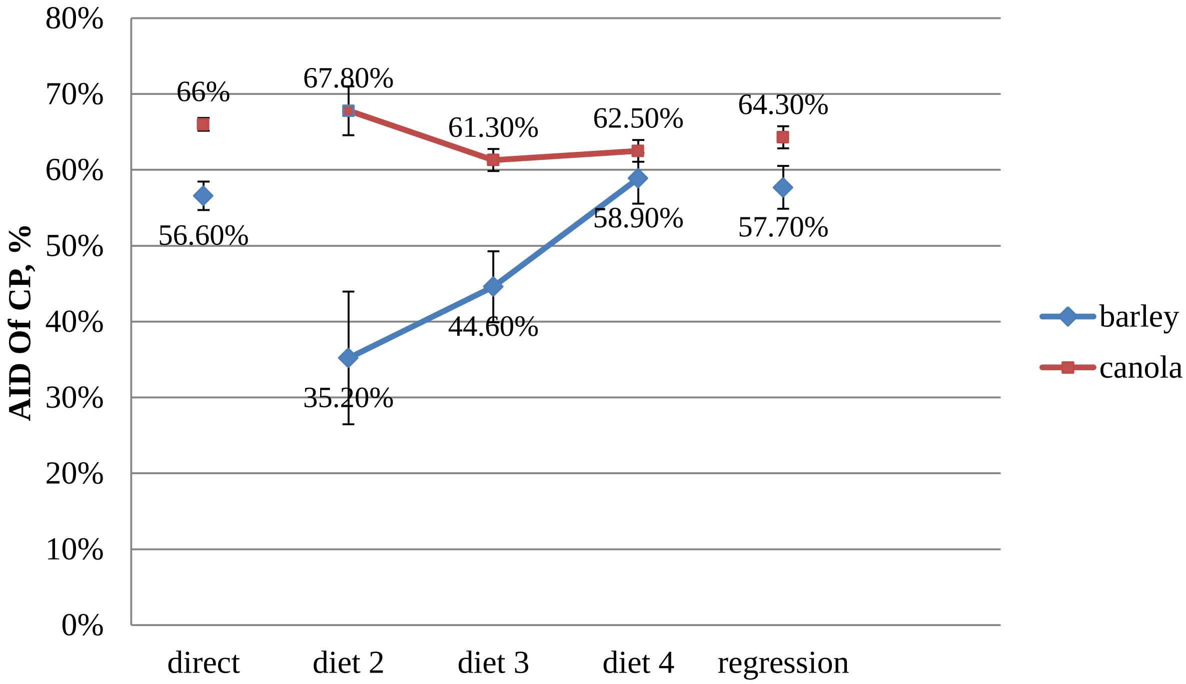
Figure 1.1 Comparison of the apparent ileal digestibility values[1] (%) of CP in barley and canola meal[2] using the direct, difference and regression method (Fan and Sauer, 1995)



[1]Means and standard errors

[2]Diet 2, 3, and 4 included 22.5 and 36.6%, 45.0 and 30.5%, and 67.5 and 24.4% barley and canola meal, respectively. The AID of CP in the respective diet was used to calculate the AID of CP in barley or canola meal using the difference method.

Table 1.1 Summary of apparent digestibility values in the test ingredient determined using different basal diets

reference	Basal diet	Test ingredient	BW, kg	Digestibility, %					Energy values (kcal/kg DM)		
				DM	N	GE	P	EE	DE	ME	NE
Stein et al., 2009[1]	corn	Corn-DDGS	71.4	75.1	84.9	75.1	56.1	72.5	4,072	3750	-
Widyaratne & Zijlstra, 2007[2]	wheat	Corn-DDGS	64.6	-	-	78.7	55.5	-	-	-	-
Widyaratne & Zijlstra, 2007	wheat	Wheat-DDGS	64.6	-	-	77.4	53.0	-	-	-	-
Nyachoti et al., 2005[2]	wheat	Wheat-DDGS	29.8	64.7	76.5	65.3	50.2	-	-	-	-
Widyaratne & Zijlstra, 2007	wheat	Wheat/corn-DDGS[3]	64.6	-	-	76.8	54.7	-	-	-	-
Nyachoti et al., 2005	wheat	Wheat/corn-DDGS[3]	29.8	66.5	74.7	67.9	55.2	-	-	-	-
Wiseman et al., 1990[4]	Complex[4]	Soybean oil	30	-	-	-	-	96.2	9,090	8,510	-
Kil et al., 2011[5]	CSBM[5]	Soybean oil[6]	22.1	-	-	-	-	95.9	-	-	4,876
Wiseman et al., 1990[4]	Complex[4]	Tallow	30	-	-	-	-	90.0	8,159	7,770	-
Kil et al., 2011[5]	CSBM[5]	CWG[5]	22.1	-	-	-	-	93.7	-	-	5,082
Montoya & Leterme, 2010	Complex[7]	Canola meal	35.6	74	81	75	-	-	3,550	-	2,430
Woyengo et al., 2010	Corn	Canola meal[8]	25.9	78.9	86.2	79.8	-	-	3,790	3,564	-
Le et al., 2012[2]	Wheat & cornstarch	Canola meal[9]	46.5	-	-	68.6	-	-	3,210	-	-
Saben et al., 1971	Wheat & SBM	SBM	33	-	89.1	-	-	-	4,120	3,870	-
Woodworth et al., 2001	Corn	SBM[10]	41	-	-	-	-	-	4,356	4,102	-
Baker & Stein, 2009	Corn	SBM[11]	38.6	-	-	-	-	-	3,827	3,620	-
May & Bell, 1971	Barley	SBM[12]	22	-	85	80	-	-	3,735	3,562	-

1. Average estimates from 4 DDGS sources.
2. Pigs were cannulated for ileal digestibility estimates
3. DDGS was produced from a mixture of wheat and corn
4. Gilts were used; basal diet included wheat meal, wheat feed, soya-bean meal, and meal and bone meal.
5. Barrows were allotted in individually pens and index method were used for estimating digestibility; CSBM: corn-soybean meal; CWG: choice white grease
6. Average estimates determined from 2 inclusion levels (5% and 10%) of soybean oil
7. Basal diet included barley, wheat, and soybean meal; regression method was used
8. Estimates determined for solvent-extracted canola meal were used
9. Average estimates determined from 2 inclusion levels of *Brassica juncea* canola meal
10. Estimates for dry extruded-expelled soybean meal with hulls were used
11. Estimates for extruded-expelled conventional soybean meal were used
12. Average estimates from 2 inclusion levels of SBM were used

Chapter 2: Evaluation of collection method on nutrient digestibility of corn-soybean meal and corn-soybean meal-dried distillers grains with solubles based diets in growing pigs

ABSTRACT

A total of 24 terminal cross-bred barrows in 2 replicates (BW = 87.9 ± 2.2 and 88.5 ± 2.6 kg, respectively) were assigned to a 2 × 2 (diet × collection) factorial arrangement of treatments to determine the effects of collection method and diet on nutrient digestibility in growing pigs. Pigs were allotted to 12 metabolism crates and provided either corn-soybean meal (CSBM) or CSBM with 20% dried distillers grains with solubles (DDGS) diet (2.8 kg/d). Diets were isocaloric and contained 0.5% of titanium dioxide (TiO_2) for estimating nutrient digestibility using the index method. After a 10-d adaptation period, total collection (TC) of feces for pigs within each diet was based on exact timing (Day collection) or the appearance of the first and second markers (carmen indigo; marker-to-marker collection, MM method) during a 4-d collection period. Urine was collected for a 4-d period in 65 mL of 6 *N* HCl daily. Subsamples of feed and feces were analyzed for DM, N, GE, and Ti, and urine for GE. The apparent total tract digestibility (ATTD) of DM and GE and energy metabolizability (ME, %) were 2.9%, 2.8% and 3.4% lower ($P < 0.05$), respectively in the DDGS vs. CSBM diet. The ATTD of N and the DE (kcal/kg) and ME (kcal/kg) were similar ($P > 0.10$) in CSBM and DDGS diets (87.79 vs. 87.53 %, 3,491 vs. 3,509 kcal/kg, and 3,381 vs. 3,368 kcal/kg, respectively). The digestibility of DM, N and GE, and DE (kcal/kg) were not different ($P > 0.10$) estimated using the different collection methods. The MM method tended to decrease ($P < 0.10$) the estimates of ME (%) and ME (kcal/kg) compared to the Day method. The ATTD of DM, N, and GE and ME (%) estimates calculated using the index method were approximately 0.5% lower ($P < 0.05$) than those using the TC method. Estimates of DE and ME (kcal/kg) were lower ($P < 0.05$) using the index vs. TC method

(3,479 vs. 3,500 kcal/kg, and 3,354 vs. 3,374 kcal/kg, respectively). Although digestibility and balance estimates were numerically lower using the MM vs. Day collection method, there was no major difference between the two collection methods.

Key words: collection method, digestibility, pig

INTRODUCTION

In vivo techniques for estimating digestibility of diets include the total collection (TC) method (i.e. the quantitative feed and feces collection method) and the index method. The TC method requires animals to be housed in the metabolism crates for the measurement of feed intake and the collection of feces and urine. The assumption of the TC method is that with timed feeding and constant daily feed intake over a sufficiently long adaptation period, daily fecal output should remain constant. The difference of nutrient inputs in the feed and outputs in the feces is used to determine apparent digestibility of nutrients in a diet or an ingredient (Adeola, 2001). Fecal collections can be determined based on two different methods. One is the “time-based” method (Day method; Liu et al., 2012), in which the fecal collection is initiated from the first meal and is ceased immediately before the last meal fed to animals during the collection period; alternatively, the marker-to-marker (MM) collection method (Pedersen et al., 2007), in which the feces belonging to a given feed is determined using an indigestible and colored marker fed in the first and last meal of the collection period. These two different strategies have been widely used by swine researchers. To date, no experiments have compared the 2 collection methods. The objective of this experiment was to determine the effects of collection method on estimating nutrient digestibility and balance in growing pigs; and, if the effect of collection method differs between diets. Additionally, the index (TiO_2) method was used to compare nutrient digestibility and balance estimates of pigs to values determined using the TC method (Kavanagh et al., 2001).

MATERIALS AND METHODS

Animals

A total of 24 terminal cross-bred barrows were used. The experiment was conducted in 2 replicates, each with 12 pigs (average BW = 87.9 ± 2.2, 88.5 ± 2.6 kg, respectively). Within each replicate, 12 pigs were randomly allotted to 4 treatments (diet × collection method combinations). Pigs were individually penned in a temperature-controlled room containing 12 metabolism crates. Pigs were fed 2.8 kg/d (~ 3.2% BW; 3.3 times of ME_m) in 2 meals at 0700 and 1700 h, respectively, and had ad libitum access to water for the entire duration of the experiment. Pigs were given 10 d to adapt to the diets and the crates, after which the specific collection methods were initiated.

Diet and Collection Method

Diets were corn-soybean meal (CSBM) or CSBM with 20% dried distillers grains with solubles (DDGS) and formulated to be isocaloric (ME basis). Formulation of the diets was based on the requirement of true ileal digestible Lys in growing-finishing pigs (NRC, 1998). The standard ileal digestible (SID) AA contents in DDGS were derived from the total AA contents of Dakoda Gold (means of 318 samples, 2010) and the average SID coefficients of AA in 10 DDGS sources (Stein et al., 2006). Also, ME of DDGS was assumed as 3,558 kcal/kg using data of Dakoda Gold. Diets contained 0.5% TiO_2 for the determination of nutrient digestibility and balance using the index method (Table 2.1). Diet samples from each experiment were collected for nutrient analysis, including DM, CP, GE, NDF and Ti.

Within each diet treatment, fecal collection of pigs was conducted using either Day or MM collection method. For the Day method, feces were collected for four-24 h

periods, which started at 0700 h on d 1 and ceased at 0700 h on d 5 of the collection period. Feed intake recording started from the morning meal of d 1 and ceased immediately before the morning meal on d 5. For the MM method, the initiation and termination of fecal collections were marked by the addition of 0.5% carmen indigo into the morning meal on d 1 and 5 of the collection period. The appearance of the first marker and second marker in the feces indicated the initiation and the termination of the collection period, respectively. During the collection period of each method, feces were collected at 0700 h and 1700 h daily and the total quantities of feces were stored at -20℃. The collection of urine was initiated on d 1 at 0700 h and ceased on d 5 at 0700 h for both collection methods. Urine was collected in the buckets containing 65 mL of 6 *N* HCl. Ten percent of the collected urine was subsampled daily in the morning and stored at -20℃ immediately after collections. At the end of the experiment, urine samples were thawed and mixed within individual pigs and subsampled.

Analytical procedures

Feces were dried in a forced-air oven (100℃), weighed, subsampled, and finely grounded through a 1.0 mm grinder before analysis. Feed and fecal samples were analyzed for DM, GE, N and Ti, and urine for N and GE. Feed samples were also analyzed for NDF. The GE of sample was determined using an adiabatic calorimeter (Parr Manual No. 153).The NDF analysis was conducted using the procedure described by Van Soest et al. (1991). The N concentration was determined by using a combustion chamber (TruSpec N Determinator, Leco Corporation, St. Joseph, MI; AOAC, 1995). The Ti analysis was based on the method described by Leone (1973).

Calculations

Digestibility of components was calculated using the index method as follows:

$$\text{Digestibility, \%} = 100 - \left[100 \times \left(\frac{[\text{TiO}_2]_{feed} \times [\text{component}]_{feces}}{[\text{TiO}_2]_{feces} \times [\text{component}]_{feed}}\right)\right]$$

(Eq. 2-1)

where $[\text{TiO}_2]_{feed}$ and $[\text{TiO}_2]_{feces}$ represent the concentration of TiO_2 in feed and feces, respectively; $[\text{component}]_{feed}$ and $[\text{component}]_{feces}$ represent the concentration of the test component in feed and feces, respectively.

ME (%), DE (kcal/kg), and ME (kcal/kg) were calculated based on the DE (%) estimated using the index method, the GE of feed and urine, and the DM intake (DMI).

$$\text{ME, \%} = \frac{\text{DE (\%)} \times \text{GE of feed} - \text{GE of urine}}{\text{GE of feed}} \times 100$$

(Eq. 2-2)

$$\text{DE, kcal/kg} = \frac{[100 - \text{DE(\%)}] \times \text{GE of feed}}{\text{DMI}}$$

(Eq. 2-3)

$$\text{ME, kcal/kg} = \frac{\text{ME(\%)} \times \text{GE of feed}}{\text{DMI}}$$

(Eq. 2-4)

Nutrient and energy digestibility and balance using the TC method were calculated as described by Adeola, 2001.

Statistics

All data were analyzed by GLIMMIX procedure. The individual pig was considered the experimental unit and a random effect. The 2 replicates were also

considered a random effect. Data from the 2 replicates were combined and analyzed as 2 × 2 factorial for the comparison of collection method and diet. To compare total collection with the index method, data were analyzed as a split-plot design, in which pigs were considered the whole plot units and the method was the split plot unit. Diet, collection (day vs. marker to marker), and method (TC vs. IN) were considered fixed effects. All means were presented as least-squares means (± SEM). P-value less than 0.05 was considered significant different; and $0.05 < P < 0.10$ was a trend.

RESULTS

Diets used in each replicate were analyzed independently. Nitrogen and GE contents were similar between the 2 replicates within each diet. The DM percentage of CSBM and the DDGS diet were 88.93 and 89.30%, respectively. The GE of DDGS diet was greater than that of CSBM diet, averaging 4,093 kcal/kg for DDGS compared to 3,944 kcal/kg for CSBM. The CP was greater in DDGS (18.19%) than in CSBM diet (14.47%). The NDF, % of DDGS diet was 2.8% greater in the first replicate than the second replicate. The average NDF concentration of DDGS diet was 4.6% greater than that of CSBM diet. The concentration of TiO_2 was approximately 0.45% in the diets. All analyzed values were similar to the calculated estimates (Table 2.2).

Digestibility estimates of nutrients and energy values within each combination of diet and collection method are presented in Table 2.3. There were no interactions ($P > 0.10$) between diet and collection method for any of the analyzed values. Digestibility of DM, energy (DE, %), and energy metabolizability (ME, %) were 2.88%, 2.77% and 3.42% greater, respectively ($P < 0.05$, SED > 0.524) in CSBM vs. the DDGS diet (88.84 vs. 85.95%; 88.50 vs. 85.72%; and 85.71 vs. 82.29%, respectively). However, no

differences ($P > 0.10$, SED = 0.610, 22.55, and 21.05, respectively) in N digestibility, DE and ME (kcal/kg) were observed between CSBM and the DDGS diets (87.79 vs. 87.53 %, 3,491 vs. 3,509 kcal/kg, and 3,381 vs. 3,368 kcal/kg, respectively).

Comparing Day to MM collection method, there were no differences ($P > 0.10$) observed for the digestibility and balance estimates of diets. Calculated estimates using the MM method were numerically decreased compared to those using the Day method. Slight tendencies ($P < 0.10$) of decreased ME (%) and ME (kcal/kg) estimates were observed using the MM vs. Day method.

The components of fecal subsamples were also used to calculate the apparent digestibility and energy estimates of diets using the index method (Adeola, 2001). Data from the index method were compared with those estimated using the TC method (Table 2.4). There were no two-way or three-way interactions ($P > 0.10$) observed in any analyzed values among diet, collection method (Day and MM), and analyzing method (TC and IN; data not shown). The ATTD of DM, N, and GE and ME (%) were approximately 0.5% lower ($P < 0.05$, SED = 0.16) estimated using the index method vs. the TC method. The DE and ME (kcal/kg) values were also lower ($P < 0.05$, SED = 6.56) calculated using the index vs. TC method (3,479 vs. 3,500 kcal/kg, and 3,354 vs. 3,374 kcal/kg, respectively).

DISCUSSION

Diet effect

Urriola and Stein (2012) reported that the ATTD of DM and GE estimated using the index method was decreased by inclusion of 30% DDGS in the CSBM in Yorkshire pigs. Consistently, increasing the dietary fiber decreased the ATTD of DM and GE

(Wilfart et al, 2007). The addition of fiber to swine diets decreases the DE concentration of the diet (Galassi et al., 2010). The concentrations of acid detergent fiber (ADF), NDF and total dietary fiber (TDF) in DDGS (9.9, 25.3, and 42.1%, respectively) are about 3 times greater than in corn (Stein, 2009). Urriola et al. (2010) reported that the ATTD of TDF in DDGS was only 46%, which may contribute to the decreased ATTD of DM and GE in these corn-based coproducts. Furthermore, dietary fiber increased the ileal flow rate of most nutrients, which subsequently decreased the apparent digestibility of carbohydrates and energy (Serena, 2008). In the current study, the DDGS diet had 4.6% greater NDF concentration than CSBM, which resulted in decreased ATTD of DM and GE and ME (%) in the DDGS vs. CSBM diet.

The different fiber concentrations of diets, however, did not affect ($P > 0.10$) the percentage of the absorbed nitrogen from dietary intake (apparent N digestibility). This result was inconsistent with some other digestibility studies. Several researchers have observed decreased protein digestibility as dietary fiber concentration increased (Wilfart et al, 2007; Urriola and Stein, 2012). A lower apparent digestibility of N can be explained by increased endogenous N losses, or decreased hydrolysis and absorption of nutrients, or both (Wilfart et al, 2007). The ileal endogenous losses (g/kg DM intake) increased with the increased level of barley bran, but was not affected by the different levels of barley hulls (Leterme et al., 2000), indicating that endogenous N loss is affected by the dietary fiber sources. Other studies showed that when fiber source does not contribute significant amounts of protein to the diet, an increase in the concentration of fiber does not affect protein digestibility (NRC, 1998). The 20% DDGS contributed 5.3% protein, representing 30% in the diet, which may help explain to the observation that nitrogen

digestibility was not different between the 2 diets in the present study. Similarly, no differences were observed for average ATTD of N in DDGS vs. corn diets (Petersen et al., 2007; Liu et al., 2012). The ATTD of CP in high fiber diet 1 (CP = 19.1%) was not different from that in the low fiber diet (CP = 14.2%); whereas, the CP digestibility was decreased in high fiber diet 2 (CP = 16.8%) vs. the low fiber diet (Serena et al., 2008). These results indicate that increased dietary CP concentration may alter the effect of dietary fiber on the endogenous N losses, resulting in the similar ATTD of CP. In the present study, the greater nitrogen intake caused increased ($P < 0.05$) digested N and N retention, respectively, during the whole collection period in the DDGS (277 g and 141 g) vs. CSBM diet (225 g and 109 g). Due to the greater N intake (g) in the DDGS diet, the apparent N digestibility and net protein utilization (NPU) were not different between the DDGS and CSBM diet ($P > 0.10$).

The estimated ME values of diets using either collection method were similar to the calculated values (3,372 kcal/kg; NRC, 1998). The analyzed ME values of CSBM and DDGS diet were not different ($P > 0.10$), averaging 3,381 vs. 3,368 kcal/kg, respectively. The similar ME estimates were most likely due to the greater GE concentration and the decreased energy metabolizability in the DDGS diet vs. CSBM diet. The ME of most practical swine diets used in North America, is 94 to 97 percent of DE (NRC, 2012). In the present study, ME percentages in DE for CSBM and DDGS diet were 97% and 96%, respectively. Petersen et al. (2007) reported that DE and ME values of DDGS and corn were not different; and they indicated that with greater CP concentration in DDGS, the increased urine N excretion may cause the decreased ME: DE ratio compared to corn.

Collection method effect

To date, few studies have directly compared the digestibility estimates of pigs using Day vs. MM method. By indirectly comparing the Day vs. MM methods, Lammers et al. (2008) reported that the apparent DE and ME of crude glycerol were not affected by different collection methods. The current study allowed the comparison of collection methods conducted in four groups of pigs fed 2 corn-soybean meal based diets, respectively. The numerically lower estimates calculated using the MM vs. Day method was likely due to the slightly different collection periods between the 2 collection methods. During the collection period, pigs fed the marker were checked 3 times / d (0700, 1300, and 1700 h, respectively) for the marker appearance. The mean transit time of the first and second markers were about 32.5 h and 32.7 h, respectively in pigs fed the CSBM diet, indicating that the collection period of pigs fed CSBM diet were similar (96 h) using the Day and MM collection methods. For the pigs fed the DDGS diet, it took about 22.8 h and 28.1 h, respectively for the 2 markers to be voided in the feces, which means the collection period of MM method was longer than 96 h (the Day method collection period). Therefore, the amount of feces excreted from pigs in MM method should be greater than those using Day method; especially, in the DDGS diet, which caused the tendency of lower digestibility estimates using the MM method. The greater fiber concentration of DDGS diet may affect the transit rate of marker through the gastrointestinal tract (GIT), which contributed to the inconsistent transit time of the 2 markers. The urine collection period was exactly the same for the pigs using different fecal collection method in this study. Therefore, the tendency of lower energy balance

estimates calculated using MM vs. Day method was due to the numerically lower digestibility estimates.

Total collection vs. index method

The lower estimates calculated using the index method compared to the total collection (TC) method were consistent with other studies. The ATTD of DM and CP were lower estimated using the index method (Cr_2O_3) vs. the MM method, mainly because the index recovery was below 100% (Mroz et al., 1996). The recovery of 0.5% TiO_2 included in the barley-wheat-SBM diet was 0.969 (Jagger et al., 1992). Kavanagh et al. (2001) reported that the ATTD of DM and GE of barley based diet evaluated with the index method (TiO_2) were lower than by the MM method; and the TiO_2 recovery rate was 92.3% (Kavanagh et al., 2001). In this experiment, the differences between average estimates of the TC method (Day and MM) and the index method were only about 0.5 percentage units for the digestibility estimates and 20 kcal/kg for dietary energy values. Significant differences were still observed because of the relatively small standard errors. The concentrations of DM, N and GE used in the index method are the same values that used in the TC method. Data were analyzed as a split-plot design, so that estimates calculated using the index and TC methods were compared within the individual pigs (whole plot unit). The variance among animals was eliminated; thus, the reduced estimates by the index vs. TC methods were primarily contributed by the differences of 5 to 6 percentage units for TiO_2 recovery relative to 100% using Day and MM methods for CSBM diet and using Day method for DDGS diet. In the current study, the TiO_2 recovery rates (calculated as described by Jagger et al., 1992) for the CSBM and DDGS diets were (94.1 vs. 94.2% and 95.3 vs. 100.7%, respectively) using Day vs. MM method. The

different index recovery rates of diets were consistent with data reported for dogs fed different diets using Cr_2O_3 as the index marker (Carciofi et al., 2007). Because the true index recovery rate is likely less than 100%, the fecal collection of pigs fed the DDGS diet using the MM method may be overestimated. Thus, the digestibility and balance estimates using the MM method for the DDGS diet may be underestimated.

CONCLUSIONS

When diets were formulated to be isocaloric, the ATTD of DM and GE and ME (%) were lower in the DDGS vs CSBM diet, due to the greater fiber concentration in the DDGS diet; whereas, N digestibility (%) was not different, which was likely a result of the relatively lower contribution of CP from the fiber source (DDGS). Energy density (DE and ME, kcal/kg) of CSBM and DDGS diet were similar, which was due to the greater GE concentration in the DDGS diet. In general, digestibility estimates using the Day and MM method were not different based on the diets and conditions. One may use either method to estimate ATTD of DM, N, and GE. Although digestibility estimates were lower using the index method vs. the TC method, the differences were not substantial. If only digestibility values are determined, the index method may be a superior choice to avoid the use of metabolism crates.

IMPLICATIONS

Results of this experiment confirmed that the dietary energy values estimated using the Day and MM method were similar for corn-soybean meal based diets. The difference of TiO_2 recovery rates calculated using the different collection methods in the DDGS diet indicated that the greater N and (or) fiber concentrations in the diet may alter the marker transit rate through the GIT of pigs. Further study may be needed to

investigate the effect of collection method using different fiber sources and (or) dietary N concentrations. Moreover, the effect of collection method on nutrient digestibility and balance of ingredients needs additional study.

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Table 2.1 Diet ingredients and nutrient composition (%, as-fed basis).

	Composition (%)			
Experiment Ingredients	CSBM[1]		DDGS[1]	
DDGS	0		20	
Corn	77.90		61.62	
Soybean meal, 47.5% CP	18.20		15.48	
Corn oil	1.10		0.35	
Limestone, ground	0.80		1.25	
Dicalcium phosphate, 18.5% P	0.80		0.10	
L-lysine-HCL	0.10		0.10	
Salt	0.25		0.25	
Vitamin premix[2]	0.20		0.20	
Trace mineral premix[3]	0.15		0.15	
TiO_2	0.50		0.50	
Calculated composition				
ME[4], kcal/kg	3,372		3,372	
CP, %	15.21		17.81	
NDF, %	9.10		14.73	
TID[4] Lys, %	0.73		0.73	
Ca, %	0.55		0.55	
aP, %	0.21		0.21	
Analyzed composition	Rep 1	Rep 2	Rep 1	Rep 2
DM, %	89.00	88.85	89.67	88.93
N, %	2.29	2.34	2.83	2.99
CP, %	14.31	14.63	17.69	18.69
NDF, %	10.72	11.23	14.22	17.01
TiO_2, %	0.49	0.44	0.45	0.43
GE, kcal/kg	3,944	3,945	4,106	4,080

[1]CSBM = corn-soybean meal; DDGS = dried distillers grains with solubles.

[2]Vitamin premix supplied per kg of diet: vitamin A (as retinyl acetate), 5500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; vitamin B_{12} (as cyanocobalamin), 33.0 mg.

[3]Trace mineral premix containing: copper (as $CuSO_4 5H_2O$), 10 mg/kg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg/kg; iron (as $FeSO_4 \cdot 2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; selenium (Na_2SeO_3), 0.3 mg/kg; zinc ($ZnSO_4 \cdot H_2O$), 125 mg/kg.

[4]ME: metabolizable energy; TID: true ileal digestibility.

Table 2.2 The effects of diet and collection method on the digestibilities of nutrients and energy values in pigs (data were analyzed only using the total collection method).

Analysis	CSBM Collection method		DDGS Collection method		Main effects[2]		
	Day	MM[2]	Day	MM	SED[1]	Diet P-value	Method P-value
Digestibilities							
DM, %	89.07	88.61	86.59	85.32	0.518	< 0.001	0.110
N, %	87.87	87.71	88.13	86.93	0.610	0.672	0.282
DE[3], %	88.66	88.34	86.45	85.00	0.555	< 0.001	0.126
ME[3], %	85.92	85.50	82.99	81.60	0.524	< 0.001	0.099
DE, kcal/kg	3,497	3,484	3,538	3,479	22.55	0.428	0.127
ME, kcal/kg	3,389	3,372	3,397	3,340	21.05	0.565	0.097
N digested, g	224.5	225.8	279.8	274.5	4.261	< 0.001	0.655
N balance, g	111.2	106.7	140.0	142.4	5.929	< 0.001	0.868
NPU[4], %	43.55	41.44	44.03	45.15	1.838	0.268	0.790
TiO_2 recovery, %	94.06	94.18	95.26	100.70	2.582	0.152	0.296

n = 6

[1]SED = Standard error of the difference.

[2]There were no interactions between diet and collection method (*P* >0.10).

[2]MM = "marker to marker" collection method.

[3]DE = energy digestibility; ME = energy metabolizibility.

[4]NPU (net protein utilization) = 100 × (N intake – N output in feces – N output in urine) ÷ N intake.

Table 2.3 The effects of approaches (total collection vs. index method) on digestibilities of nutrients and energy values in pigs

Analysis	Approaches		SED[1]	Main effect of approach P-value
	Total collection	Index method		
Digestibilities				
DM, %	87.40	86.91	0.157	0.006
N, %	87.66	87.15	0.160	0.005
DE, %	87.11	86.60	0.164	0.006
ME, %	84.00	83.49	0.163	0.006
DE, kcal/kg	3,500	3,479	6.56	0.006
ME, kcal/kg	3,374	3,354	6.56	0.006

n = 24

[1]SED = Standard error of the difference.

Chapter 3: Digestible and metabolizable values of dried distillers grains with solubles (DDGS) estimated by corn-soybean meal or barley-canola meal as the basal diet using different collection methods

ABSTRACT

An experiment was conducted to determine the effects of collection method and basal diet on estimating digestibility of dried distillers grains with solubles (DDGS). A total of 24 terminal cross-bred barrows in 2 replicates (BW = 90.3 ± 2.1, 90.9 ± 2.4 kg, respectively) were randomly assigned to metabolism crates and fed 1 of the 4 diets: corn-soybean meal diet (CSBM, basal 1), 20% of basal 1 replaced by DDGS (total 1), barley-canola meal diet (BCM, basal 2), and 20% of basal 2 replaced by DDGS (total 2). The apparent digestibility and energy values of diets were estimated using the time-based collection (Day) and marker-to-marker collection (MM) methods, which were conducted within each individual pig by separate collections and measurements of feces. Urine was collected for a 4-d period in 65 mL of 6 *N* HCl daily. Subsamples of feed and feces were analyzed for DM, N, GE, and Ti, and urine for GE. The apparent total tract digestibility (ATTD) of DM, CP, GE and the DE and ME in DDGS based on 2 basal diets were calculated using the difference method by subtracting the nutrient and energy contribution of basal diet from the respective total diet. The ATTD of DM, CP, and GE and the apparent DE and ME values for CSBM based diets were greater ($P < 0.05$) than those for BCM based diets. There were no interactions ($P > 0.10$) between basal diet and collection method. Comparing basal 1 with basal 2, no differences ($P > 0.10$) were observed on apparent digestibility and energy estimates of DDGS. Digestibility estimates of diets and DDGS calculated using Day and MM methods were not different ($P > 0.10$). The average DE and ME values (as-fed basis) of DDGS were 4,035 and 3,704 kcal/kg and 4,081 and 3,651 kcal/kg, respectively estimated using basal 1 and basal 2 diets. In

conclusion, different collection methods did not affect estimation of digestibility values; also, basal diets may not affect digestibility estimates of DDGS.

Key words: collection method, basal diet, DDGS, digestibility, pig

INTRODUCTION

Diets for swine are formulated by mixing feed ingredients to meet the nutrient and energy requirements (NRC, 2012). Therefore, it is important to estimate the digestibility of each ingredient in the diet and use that to predict the nutrient values of diets. Ingredient digestibility can be determined by direct or difference methods (Fan and Sauer, 1995). Using the direct method, the diet is formulated primarily with the test feedstuff so that all the interested components are contributed by the test ingredient. The direct method is commonly used for ingredients with high feeding values (cereal grains; Pedersen et al., 2007b; Urriola et al., 2009). In other cases, the test feedstuff is not able to be used as the major ingredient to supply all the interested components or has poor palatability. The diets are therefore formulated with both the test feedstuff and other feedstuffs (basal diet), which also supply the interested components and this is known as the difference (indirect) method (Kil et al., 2011). This method assumes that there are no interactions between the digestibility values of a component in the test feedstuff and the basal diet (Adeola, 2001); however, this assumption may not be true. The digestibility estimates of a test feedstuff may be different if the basal diets containing dramatically different nutrient compositions are used. To date, different basal diets, such as corn, corn-soybean meal, wheat, barley, etc. have been used to investigate the digestibility values of ingredients; nevertheless, few studies have compared the effects of using different basal diets (Stein et al., 2009; Kil et al., 2011; Widyaratne & Zijlstra, 2007; May & Bell, 1971). The objective of this study is to compare the digestibility estimates of DDGS calculated using a corn-soybean meal vs. barley-canola meal basal diets. In addition, the previous study has shown that using the Day and MM collection method, no major differences

were found for the digestibility estimates of CSBM based diet (Chapter 2). In this study, the Day and MM were compared relative to estimating the digestibility values of DDGS using different basal diets.

MATERIALS AND METHODS

Animals

A total of 24 terminal cross-bred barrows were used. The experiment was conducted in 2 replicates, each with 12 pigs (average BW = 90.3 ± 2.1, 90.9 ± 2.4 kg, respectively). Within each replicate, 12 pigs were individually penned in a temperature-controlled room containing 12 metabolism crates and given 1 of the 4 diets (described below). Pigs were adapted to diets and crates for 9 d, after which the collection period (~ 4 d) was initiated. The feed intake of all pigs were adjusted to 2.4 kg/d (~ 2.7% BW) during the early adaptation period according to the lowest feed intake and remained constant during the remainder of the experimental period. Animals were fed twice daily at 0700 and 1700 h, respectively, and had ad libitum access to water for the entire duration of the experiment.

Diet and Collection Method

Diets (Table 3.1) were based on corn-soybean meal (CSBM; basal 1) and 20% of basal 1 replaced by DDGS (total 1), or barley-canola meal (BCM; basal 2) and 20% of basal 2 replaced by DDGS (total 2). Digestibility values of DDGS were estimated using the difference method based on the digestibility estimates of 2 groups of pigs fed basal 1 and total 1 or basal 2 and total 2 diets (Adeola, 2001); details will be described in the calculation section. Diet formulation was based on the requirement of SID Lys in growing-finishing pigs (NRC, 2012). Diets contained 0.5% of titanium dioxide (TiO_2) for

estimating digestibility using the index method, but data are not presented at this time. Diet samples from each experiment were collected for nutrient analysis, including DM, CP, GE, and NDF (Table 3.2).

Individual pig was the experimental unit, and the Day and MM collection methods were administered on each individual pig (see Figure 3.1). Day collection was initiated at 0700 on d 1 of the collection period; meanwhile, the respective meals mixed with 0.5% marker (carmen indigo) were fed for all the pigs. Feces excreted from the beginning of Day collection to the appearance of the first marker were collected in Bag-1. At 0700 h on d 5, Day collection was ceased and the second marked meal was fed. Feces excreted from the appearance of the 1st marker to the end of Day collection were collected into Bag-2. Feces excreted after that until the appearance of the 2nd marker were collected in Bag-3. The sum of the amount of feces in Bag-1 and Bag-2 represented fecal collection using the Day method; and the addition of Bag-2 and Bag-3 represented fecal collection using the MM method. Fecal samples were stored at -20℃ immediately after collections.

During the collection period, feces were collected at 0700 and 1700 h daily and the collection of urine was initiated on d 1 at 0700 h and ceased on d 5 at 0700 h. Urine was collected in buckets containing 65 mL of 6 *N* HCl. When the experiment started, the plan was to store 10% of the collected urine in the -20℃ freezer. However, the volume of urine that some pigs excreted decreased relative to expectations in this study, therefore a constant amount (10% to 50%) of the daily collected urine was stored for the individual

pigs. At the end of the experiment, urine samples were thawed, pooled within individual pigs, and subsampled.

Analytical procedures

Feces in each bag were dried in a forced-air oven (100℃) separately, weighed, subsampled and finely grinded through a 1-mm grinder prior to analysis. Fecal subsamples from each bag were analyzed for DM, GE and N. Subsequently, data of Bag-1 and Bag-2 were pooled for determining digestibility values using the Day method; and data of Bag-2 and Bag-3 were pooled for using the MM method. Feed samples were analyzed for DM, GE, N, and NDF. Urine was analyzed for GE and used for calculating ME values. Gross energy was analyzed using an adiabatic calorimeter (Parr Manual No. 153). The analysis of NDF was conducted using the procedure described by Van Soest et al. (1991). The N concentration was determined by using a combustion chamber (TruSpec N Determinator, Leco Corporation, St. Joseph, MI; AOAC, 1995).

Calculations

Nutrient and energy digestibility and balance of each diet using the total collection method (Day or MM) were calculated based on the examples described by Adeola (2001) and those in the previous chapter. By subtracting the contribution of basal diet to the respective total diet, the digestibility and energy values of DDGS were calculated using the difference method (Baker and Stein, 2009). In this study, the average estimates of basal diet in each replicate (n = 3) were used to calculate the digestibility and energy values of DDGS in the total diet for pigs in the same replicate (Fan and Sauer,

1995). The energy values of diets and DDGS were calculated on a DM basis, and subsequently converted to an as-fed basis (based on the analyzed DM).

Statistics

All data were analyzed by GLIMMIX procedure. The experiment was a completely randomized design, in which the individual pig was considered the experimental unit and a random effect. The 2 replicates were also considered a random effect. Data from the 2 replicates were combined and analyzed as a split-plot design. Pigs that were assigned 1 of the 4 diets or 1 of the 2 total diets when comparing DDGS estimates were the whole plot unit, and the collection methods (Day and MM) were the split plot units. All means were presented as least-squares means (± SEM). Observations from 1 pig fed the basal 2 diet were not used because of feed refusal and was considered an outlier (Kerr et al., 2009). P-value less than 0.05 was considered significant difference; and $0.05 < P < 0.10$ was a trend.

The statistic model is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \omega_{ik} + s_{ijk}$$

where Y_{ijk} is the individual observations; μ is the average digestibility estimates of diet or DDGS; α_i is the effect of diet or basal diet; β_j is the effect of the collection method; $(\alpha\beta)_{ij}$ is the interaction of diet and collection method; γ_k is the variance due to replicates; ω_{ik} is the variance among animals; and s_{ijk} is the residual error.

RESULTS

The DDGS and 4 diet samples from 2 replicates were analyzed for nutrient composition (Table 3.2). The DM concentrations in DDGS, CSBM based diets, and BCM

based diets were 91.31, 89.55, and 92.27%, respectively. The DDGS contained greater CP concentration (27.30%) than the basal 1 (16.00%) and basal 2 (22.34%) diets, which increased the CP concentrations in the total 1(18.46%) and total 2 (23.75%) diets. The GE content of DDGS was 4,808 kcal/kg, which was also greater than that of basal 1 (3,953 kcal/kg) and basal 2 (4,246 kcal/kg) diets. The NDF of DDGS (37.71%) was greater than that of basal 1 (15.49%) and total 1 (18.05%), but was similar to basal 2 (35.11%) and total 2 (34.11%) diets.

The apparent digestibility and energy values of 4 diets estimated using different methods are shown in Table 3.3. There were no interactions ($P > 0.10$) between diet and method for all estimated responses. The ATTD of DM, CP, and GE, ME, %, and the apparent DE and ME values for CSBM based diets were greater ($P < 0.05$) than those for BCM based diets. Estimates calculated using Day and MM method were not different ($P > 0.10$). The apparent digestibility values of basal 1 vs. total 1 and basal 2 vs. total 2 were not different ($P > 0.10$). Digestible energy and ME (kcal/kg of DM) in basal 1 (3,921 and 3,798, respectively) and total 1 diet (4,046 and 3,886, respectively) were not different ($P > 0.10$); whereas, those ME values tended to be greater ($P < 0.10$) in total 2 (3,501 and 3,312, respectively) vs. basal 2 diets (3,303 and 3,146, respectively).

There were no interactions ($P > 0.10$) between diet and collection method relative to the N balance data (Table 3.4). All estimates differed ($P < 0.05$) among diets; however, estimates calculated using the Day method were not different ($P > 0.10$) from using MM method. Nitrogen intake, fecal N output, and urinary N output were greater ($P < 0.05$) for pigs fed BCM vs. CSBM based diets; these values were not different ($P > 0.10$) in basal 1 vs. total 1 and basal 2 vs. total 1 except that N intake of pigs fed total 1 were greater ($P <$

0.05) than those fed basal 1 diet. Pigs fed the basal diets absorbed and retained less (*P* < 0.05) N than pigs fed the respective total diets; whereas, the quantities of N absorbed and N retained by pigs fed basal 1 vs. basal 2 were not different (*P* > 0.10), as well as total 1 vs. total 2 (*P* > 0.10). The retention rates of N were greater (*P* < 0.05) for pigs fed CSBM vs. BCM based diets.

The apparent nutrient digestibility and energy values determined in DDGS did not show an interaction (*P* > 0.10) between basal diet and collection method (Table 3.5). The ATTD estimates of DM, CP, and GE were not affected (*P* > 0.10) by using different basal or collection methods. Additionally, DE and ME for DDGS were not different (*P* > 0.10) estimated using different basal diets or collection methods. The average DE and ME values (as-fed basis) of DDGS were 4,035 and 3,704 kcal/kg, and 4,081 and 3,651 kcal/kg, respectively estimated using basal 1 and basal 2 diet. Similarly, N balance data were not different (*P* > 0.10) estimated using basal 1 vs. basal 2 or using Day vs. MM methods (Table 3.6).

DISCUSSION

Nutrient digestibility and energy values of diets

The ME (kcal/kg as-fed) values of CSBM based diets (3,397 and 3,485, respectively) were greater than the predicted NRC (2012) values (3,290 and 3,319, respectively); whereas, ME values for BCM based diets (2,905 and 3,053, respectively) were lower than the NRC values (3,071 and 3,144, respectively). This may due to the different nutrient availability of various ingredient sources. The ATTD of DM, and GE and ME (%) for CSBM based diets were similar as the previous study (Chapter 2);

whereas, likely due to the lower FI (Haydon et al., 1984), the apparent CP digestibility estimates were approximately 2% greater than the previous study (Chapter 2).

The consistently lower digestibility values of BCM vs. CSBM based diets were most likely due to the greater NDF concentrations of the BCM diets. The ATTD of GE in canola meal was 60.3 to 68.6% depending on different NDF contents of varieties (Le et al., 2012). Rapeseed meals (canola meal) were shown to have lower apparent digestibility and energy values than soybean meal (May and Bell, 1971; Saben et al., 1971). Results of the current study are in agreement with studies that showed the inclusion of canola meal decreased the apparent digestibility of diets (Sanjayan, 2013). Barley contains greater CP but also greater fiber content (ADF and NDF) than yellow dent corn (Pedersen et al., 2007b), which may be one of the factors for the decreased digestibility estimates using BCM diets.

The differences between the CSBM and CSBM-DDGS diet that were seen in the previous study were not observed in the current study, but the numerically decreased digestibility estimates except for CP were consistent with the previous study (Chapter 2). The average ATTD coefficient of CP in 4 DDGS sources was 85% (Stein et al., 2009), which was more digestible than canola meal and less digestible than barley (May and Bell, 1971). The superior availability of CP in DDGS compared to canola meal may result in numerically greater ATTD of CP in total 2 vs. basal 2 diets. The 4 diets were not formulated to be isocaloric (ME basis) such that the total diets were expected to be greater in DE and ME than the respective basal diets. In the present study, the supplementation of DDGS numerically increased DE and ME for CSBM diet and tended to increase DE and ME for BCM diets.

The similar digestibility estimates using Day and MM methods in both CSBM and BCM diets were consistent with the previous study for CSBM-based diets (Chapter 2). Similarly, the apparent DE and ME of crude glycerol were not affected by the different collection methods (Day vs. MM; Lammers et al., 2008). It should be noted that the digestibility estimates for basal 2 diet using Day method were approximately 2% greater than using MM method; whereas, differences of means for the other 3 diets were less than 0.5%. This difference was likely due to less amounts of fecal collection by Day (1,977 g) vs. MM (2,146 g) methods for pigs fed basal 2 diet.

Substitution of 20% of DDGS increased N intake but did affect the fecal and urinary N outputs; therefore, the amounts of N absorbed and retained by pigs were increased by the inclusion of DDGS in the total vs. basal diets. However, when calculated on a percentage basis, N retention was not affected by DDGS substitution.

Nutrient digestibility and energy values of DDGS

Using basal 1, the ATTD estimates of DM, CP, and GE and DE and ME values of DDGS were slightly greater than the upper ranges of means of 14 DDGS sources (70.6 ~ 78.2%, 77.1 ~ 88.4%, 71.1 ~ 82.8%, 3,446 ~ 3,957 kcal/kg, and 3,226 ~ 3,738 kcal/kg, respectively; Pedersen et al., 2007a; Stein et al., 2009). Nevertheless, the GE of DDGS used in the current study (4,808 kcal/kg) was greater than the highest value (4,324 kcal/kg) among the 14 DDGS sources previously cited. When basal 2 were fed, the observations were more variable, which was likely due to relatively greater NDF concentrations that may alter the digestive processes (Wilfart et al., 2007).

Although there were no significant differences between Day and MM method, the ATTD of DM, CP, and GE in DDGS estimated using basal 2 were 7.4, 6.0, and 6.7 %,

respectively lower using the Day vs. MM methods. This was mostly likely due to the greater digestibility estimates of basal 2 calculated using Day vs. MM methods, resulting in underestimation of nutrient and energy values contributed by DDGS using the Day method.

Differences between the nutrient values of DDGS using different basal diets were not significant, which was likely due to the relatively low inclusion level of DDGS that exaggerated the standard error of the estimates. Fan and Sauer (1995) suggested that using the difference method, increasing the inclusion level of the test ingredient in the total diet decreased the standard errors of the estimates. In addition, the DE and NE of canola meal were numerically lower estimated at inclusion level of 25% than at 50% (Le et al., 2012). In the current study, the average estimates using the Day method were numerically greater by basal 1 vs. basal 2; whereas, means using basal 1 were numerically lower than using basal 2 by the MM method. Similarly, different ATTD of CP, DE, and ME in SBM were reported using diets composed of wheat and SBM vs. barley as the basal diets (Saben et al., 1971; May and Bell, 1971). The difference of CP contributions to the total diet between the basal diet and the test feedstuff may affect the digestibility estimates of the ingredient (May and Bell, 1971). Moreover, the greater NDF concentration in the BCM based diets may affect the nutrient composition in the excreta. Dietary fiber increased endogenous and exogenous losses (Schulze et al., 1994; Leterme et al., 2000). It was possible that the endogenous and exogenous losses due to the substitution of DDGS were underestimated, subsequently affecting the determination of DDGS digestibilities using basal 2.

CONCLUSIONS

The apparent nutrient digestibility and energy values of BCM based diets were systematically lower than those of CSBM based diets due to greater fiber concentrations. There were no major differences between the Day and MM collection methods for the determination of diet and DDGS digestibility estimates. No significant differences were detected for digestibility of DDGS using different basal diets. It appears that either collection method can be used to estimate digestibility values of diets and DDGS when using CSBM basal diet.

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Table 3.1 Ingredient composition (%, as-fed basis) and calculated nutrient composition of diets

Ingredient	Basal 1	Total 1	Basal 2	Total 2
Corn	76.83	61.46	-	-
Soybean meal, 47.5% CP	20.50	16.40	-	-
Barley	-	-	68.35	54.68
Canola meal	-	-	29.50	23.60
DDGS[1]	-	20.00	-	20.00
L-Lysine-HCl, 78%	0.15	0.12	0.15	0.12
Dicalcium phosphate, 18.5% P	0.65	0.52	0.30	0.24
Limestone	0.77	0.62	0.55	0.44
Salt	0.25	0.20	0.25	0.20
TM[2]	0.15	0.12	0.15	0.12
Vitamin[3]	0.2	0.16	0.2	0.16
TiO_2	0.5	0.40	0.5	0.40
Calculated nutrient composition				
ME[4] (kcal/kg)	3,290	3,319	3,071	3,144
Fermentable fiber (%)	10.45	14.06	11.71	15.07
CP (%)	16.26	18.47	19.97	21.45
Ca (%)	0.52	0.44	0.52	0.44
STTD[5] of P (%)	0.24	0.28	0.24	0.28
SID[5] AA (%)				
Arg	0.91	0.92	0.93	0.93
His	0.39	0.42	0.46	0.48
Ile	0.57	0.61	0.50	0.55
Leu	1.29	1.56	0.94	1.28
Lys	0.80	0.73	0.80	0.73
Met	0.24	0.28	0.28	0.31
Met + Cys	0.47	0.54	0.58	0.63
Phe	0.69	0.77	0.61	0.71
Phe + Tyr	1.13	1.29	0.98	1.17
Thr	0.49	0.53	0.50	0.54
Trp	0.16	0.16	0.14	0.14
Val	0.64	0.71	0.67	0.74

[1]DDGS = dried distillers grains with solubles.

[2]Trace mineral premix containing: copper (as $CuSO_45H_2O$), 10 mg/kg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg/kg; iron (as $FeSO_4 \cdot 2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; selenium (Na_2SeO_3), 0.3 mg/kg; zinc ($ZnSO_4 \cdot H_2O$), 125 mg/kg.

[3]Vitamin premix supplied per kg of diet: vitamin A (as retinyl acetate), 5500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; vitamin B_{12} (as cyanocobalamin), 33.0 mg.

[4]ME: metabolizable energy

[5]STTD: standardized total tract digestible; SID: standard ileal digestibility

Figure 3.1 Fecal collection outlines

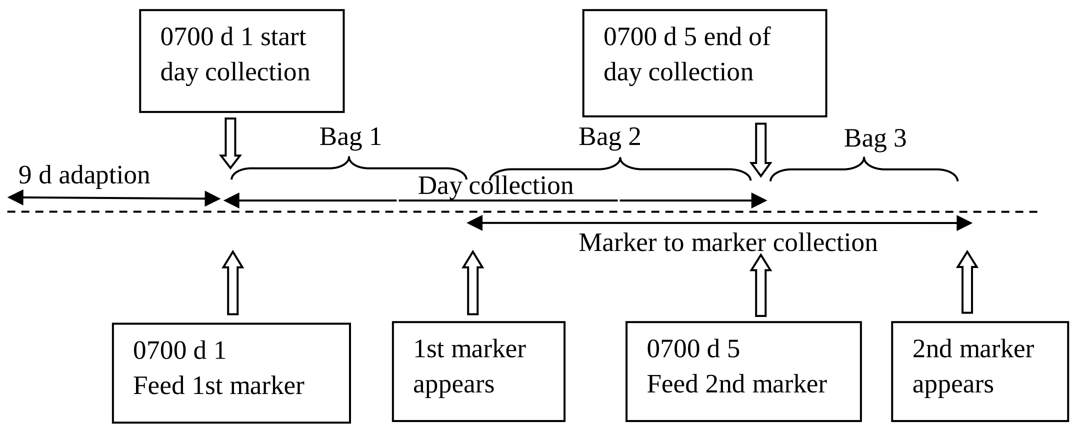

Table 3.2 Analyzed composition of diets (as-fed basis).

		Basal 1		Total 1		Basal 2		Total 2	
Analysis	DDGS	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
DM, %	91.31	88.91	89.97	89.62	89.70	92.27	92.47	92.13	92.22
CP, %	27.30	15.71	16.28	18.57	18.35	21.93	22.75	23.76	23.73
NDF, %	37.71	14.28	16.69	17.69	18.40	36.52	33.69	36.10	32.11
GE, kcal/kg	4,808	3,930	3,976	4,153	4,126	4,252	4,239	4,369	4,347

Table 3.3 The effects of diet and collection method on the determination of digestibilities of nutrients and energy values in pigs

		Corn-SBM		Barley-Canola meal		SED		P-values		
Responses	method	0% DDGS[1]	20% DDGS[1]	0% DDGS[2]	20% DDGS[1]	Diet	Method	Diet	Method	Diet × Method
digestibilities										
DM, %	DAY	88.49	87.12	75.00	75.27	1.537	0.375	0.008	0.525	0.146
	MM	88.50	87.31	73.25	75.44					
CP, %	DAY	89.29	89.39	69.28	72.89	2.240	0.587	0.009	0.548	0.344
	MM	88.98	89.34	67.47	73.04					
DE, %	DAY	88.70	87.54	72.77	73.95	1.654	0.399	0.006	0.542	0.170
	MM	88.70	87.72	70.98	74.16					
ME, %	DAY	85.93	84.08	69.36	69.95	1.420	0.399	0.004	0.542	0.171
	MM	85.93	84.27	67.57	70.16					
DE, kcal/kg as-fed	DAY	3,507	3,624	3,088	3,223	80.9	16.5	0.019	0.531	0.169
	MM	3,506	3,631	3,012	3,232					
DE, kcal/kg of DM	DAY	3,921	4,041	3,344	3,496	80.1	18.2	0.009	0.538	0.175
	MM	3,920	4,050	3,261	3,506					
ME, kcal/kg as-fed	DAY	3,397	3,481	2,943	3,048	71.2	16.5	0.012	0.531	0.169
	MM	3,397	3,488	2,866	3,057					
ME, kcal/kg of DM	DAY	3,798	3,882	3,187	3,307	69.1	18.2	0.006	0.538	0.175
	MM	3,798	3,890	3,105	3,317					

[1]n = 6 for the 2 groups of pigs that fed corn-SBM based meal and barley-canola meal with DDGS
[2]n = 5 for those fed the barley-canola basal diet.
All the means for CSBM based diets were significantly greater ($P < 0.05$) than those for BCM based diets regardless of method.

Table 3.4 The effects of diet and collection method on the nitrogen balance values in pigs, DM basis.

		Corn-SBM		Barley-Canola meal		SED		P-values		
Responses	method	0% DDGS[1]	20% DDGS[1]	0% DDGS[2]	20% DDGS[1]	Diet	Method	Diet	Method	Diet × Method
N intake, g	DAY & MM	245.2[a]	279.9[b]	336.0[c]	353.1[cd]	7.43	-	< 0.001	-	-
N in feces, g	DAY	26.3[a]	29.7[a]	103.4[b]	96.5[b]	8.93	1.66	0.011	0.539	0.282
	MM	27.0[a]	29.8[a]	109.4[b]	95.5[b]					
N in urine, g	DAY & MM	77.5[a]	89.4[ab]	91.5[b]	94.5[b]	9.02	-	0.073	-	-
N absorbed, g	DAY	218.9[a]	250.2[bc]	231.8[ab]	256.6[c]	9.43	1.62	0.039	0.517	0.282
	MM	218.1[a]	250.1[bc]	225.7[ab]	257.6[c]					
N retained, g	DAY	141.4[a]	160.8[b]	141.0[a]	162.1[b]	8.73	1.64	0.015	0.529	0.282
	MM	140.7[a]	160.7[b]	135.0[a]	163.1[b]					
N retention, %	DAY	57.7[a]	57.5[a]	42.0[b]	46.0[b]	3.20	0.59	0.027	0.549	0.344
	MM	57.5[a]	57.5[a]	40.2[b]	46.2[b]					

[1]n = 6 for the 2 groups of pigs that fed corn-SBM based meal and barley-canola meal with DDGS
[2]n = 5 for those fed the barley-canola basal diet
Means within a row not sharing a common superscript letter (a to c) are significantly different ($P < 0.05$), for results within the same collection method and response. Responses represent the total amounts of the collection period (4 d), respectively.

Table 3.5 The effects of basal diet and collection method on the digestibility of nutrients and energy values of DDGS in pigs, as-fed basis.

		DDGS[1]		SED		P-values		
Responses	Method[2]	Basal 1	Basal 2	Basal diet	Method	Basal diet	method	Basal × method
digestibilities								
DM, %	Day	81.89	77.81	11.25	2.36	0.950	0.113	0.194
	MM	82.70	85.18					
CP, %	Day	89.24	84.63	15.74	2.80	0.915	0.266	0.353
	MM	89.82	90.65					
GE, %	Day	83.52	79.36	10.69	2.34	0.921	0.140	0.235
	MM	84.32	86.07					
DE, kcal/kg as-fed	Day	4,016	4,024	611	139	0.946	0.597	0.790
	MM	4,054	4,138					
DE, kcal/kg of DM	Day	4,398	4,179	563	123	0.921	0.140	0.235
	MM	4,440	4,532					
ME, kcal/kg as-fed	Day	3,685	3,490	548	112	0.932	0.140	0.234
	MM	3,723	3,812					
ME, kcal/kg of DM	Day	4,035	3,822	600	123	0.932	0.140	0.235
	MM	4,077	4,175					

[1]n = 6; Basal 1: corn-soybean meal; Basal 2: barley-canola meal
[2]Day: time-based collection method; MM: marker-to-marker collection method