RELATIONSHIP BETWEEN DIGESTIBILITY INDEX MARKER AND DIETARY CHARACTERISTICS IN THE DETERMINATION OF ENERGY AND NUTRIENT UTILIZATION FOR PIGS AND BROILER CHICKENS

by

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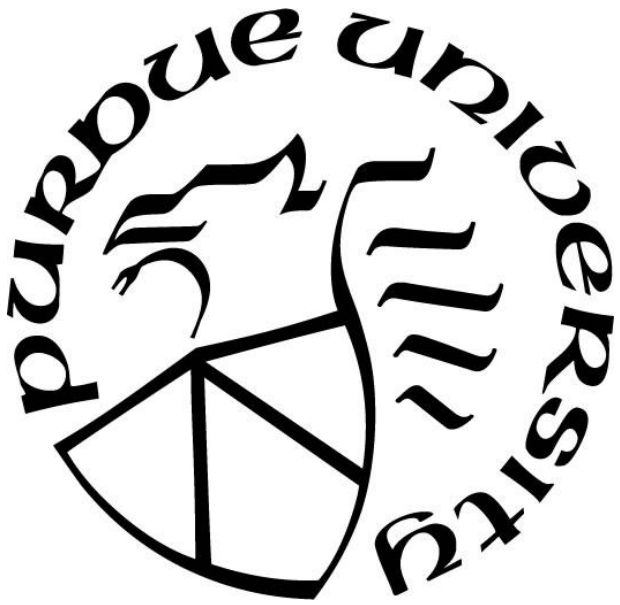

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This dissertation is dedicated to my beloved parents, sister, and grandparents.

Your love has been the major spiritual support in my life.

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LIST OF ABBREVIATIONS

SYMBOL	DESCRIPTION
AA	Amino acids
AIA	Acid insoluble ash
AID	Apparent ileal digestibility
ATTD	Apparent total tract digestibility
ATTU	Apparent total tract utilization
BEL	Basal ileal endogenous losses
CB	Corn bran
CM	Canola meal
CP	Crude protein
Cr_2O_3	Chromic oxide
CS	Corn starch
CSBM	Corn-soybean meal-based
DAE	Diatomaceous earth
DDGS	Sorghum distillers' dried grains with solubles
DE	Digestible energy
DIM	Digestibility index marker
DM	Dry matter
FI	Feed intake
FME	Formulation method for energy
GE	Gross energy
IRA	Ileo-rectal anastomosis
MBM	Meat and bone meal
ME	Metabolizable energy
N	Nitrogen
NE	Net energy
NFD	Nitrogen-free diet
NSP	Non-starch polysaccharide

OB	Oat bran
PC	Positive control
PVTC	Post-valve T-caecum
SED	Standard error of difference
SICV	Steered ileo-cecal valve
SID	Standardized ileal digestibility
TEL	Total ileal endogenous losses
TID	True ileal digestibility
TiO_2	Titanium dioxide
TP	Time period
WB	Wheat bran

ABSTRACT

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Title: Relationship Between Digestibility Index Marker and Dietary Characteristics in the Determination of Energy and Nutrient Utilization for Pigs and Broiler Chickens
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The objective of this study was to investigate the effect of type and level of digestibility index marker (DIM) and dietary characteristics including dietary fiber type, dietary protein sources, and inclusion of xylanase in pigs and broiler chickens.

An experiment was conducted to investigate if (i) the apparent digestibility of gross energy (GE) and nitrogen (N) were influenced by the type of DIM and dietary fiber; (ii) the concentration pattern of DIM was influenced by dietary fiber, ileal digesta collection day (Day), and time period (TP). Eighteen barrows (initial BW = 24.2 ± 0.3 kg) fitted with a T-cannula at the end of the ileum were used in a 2-period study. Three corn-soybean meal-based diets were formulated with corn starch, corn bran or oat bran at 100 g/kg. Acid insoluble ash (AIA), chromic oxide (Cr_2O_3), and titanium dioxide (TiO_2) were included as DIM in each diet. Each period consisted of a 7-d adjustment period followed by a 3-d total fecal collection period and a 3-d ileal digesta collection period, where ileal digesta was collected every 3 h between 09:00 to 21:00 h with 4 TP on each of the 3 day. The DIM had similar effect on apparent ileal digestibility (AID) of GE and N within each diet, but different effects among the 3 diets. However, the apparent total tract digestibility (ATTD) of GE or N of corn starch and the ATTD of N of corn bran determined by the 3 DIM were not different. The recovery of TiO_2 in feces of pigs fed the oat bran was 78.3%, which was the least among the 3 diets ($P < 0.05$). The distribution of Cr concentration in ileal digesta of pigs fed cornstarch, corn bran, and oat bran was similar to that of Ti and AIA irrespective of TP. In

conclusion, the AID of GE or N was more influenced by the choice of DIM compared with ATTD; the recovery of TiO_2 in pigs fed oat bran was less than corn starch or corn bran; the Day had limited effect on DIM concentration; and the three DIM moved synchronously in diets irrespective of TP.

Another study was conducted to investigate if the AID of GE or N was influenced by inclusion level and type of DIM and inclusion level of OB, and if the ATTD of GE or DIM recovery was influenced by the three aforementioned factors and duration of feces collection. Six diets were formulated as a 2 × 3 factorial arrangement with two levels of OB (0 or 100 g/kg) and three levels of DIM (2.5, 5.0, or 7.5 g/kg). Both Cr_2O_3 and TiO_2 were added to the same diet as DIM and their inclusion levels were consistent in each experimental diet. In Exp. 1, eighteen barrows (initial BW = 24.2 ± 0.3 kg) fitted with T-cannulas at the distal ileum were used in a triplicate 6 × 2 incomplete Latin Square design with 6 dietary treatments and 2 periods. The ileal digesta were collected for 3 d after 5-d adaptation. In Exp. 2, a total of 72 barrows (initial BW = 26.9 ± 0.5 kg) were used in a randomized complete block design, and the feces were collected for either 3 or 5 d after a 7-d adaptation according to the assignment. Experimental diets were same as Exp. 1. The AID of GE and N determined by TiO_2 were greater ($P < 0.05$) than Cr_2O_3 regardless of the OB level and DIM level. Neither the OB level nor the DIM level affected the AID of GE or N. The DIM level and duration of feces collection had no effect on ATTD of GE and DIM recovery. The ATTD of GE were greater ($P < 0.05$) determined by TiO_2 than that determined by Cr_2O_3. Similarly, the recovery of TiO_2 was greater ($P = 0.007$) than Cr_2O_3. Inclusion of 100 g/kg OB did not affect the recovery of DIM. In conclusion, the AID of GE and N, the ATTD of GE, and the recovery of Cr_2O_3 or TiO_2 were affected by DIM type, but not DIM level; the inclusion of OB had no effect on AID of GE and N, and DIM recovery; and the duration of feces collection had no effect on ATTD of GE, and DIM recovery.

The additivity of AID and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in mixed diets containing wheat, canola meal (CM), meat and bone meal (MBM), and sorghum distillers' dried grains with solubles (DDGS) fed to pigs with Cr_2O_3 and TiO_2 as DIM was investigated in the third study. Four diets were prepared to contain wheat, CM, MBM, or DDGS as a sole source of N; three mixed diets were prepared to contain wheat, CM, and MBM; wheat, MBM, and DDGS; or wheat, CM, MBM, and DDGS; and a N-free diet was prepared to estimate the BEL of CP and AA. Both Cr_2O_3 and TiO_2, each at 5 g/kg were incorporated into each diet. Sixteen barrows (initial BW = 34.7 ± 0.6 kg) surgically fitted with T-cannulas at the distal ileum were allotted to a duplicate 8 × 4 incomplete Latin square design with 8 experimental diets and 4 periods. Chromic oxide and TiO_2 determined similar BEL, AID, and SID of CP and AA. In wheat-CM-MBM diet, the measured AID of CP and most AA determined with Cr_2O_3 or TiO_2 were not different from the predicted values. The results indicated that the determination of BEL, AID, and SID of CP and AA were not affected by DIM type; the additivity of AID and SID of CP and most indispensable AA in mixed diets was not affected by DIM type; and more accurate prediction of ileal digestibility of AA was achieved using SID rather than AID in mixed diets containing wheat, CM, MBM, and DDGS.

The aim of the last study was to investigate the growth performance and nutrient utilization responses of broiler chickens and the nutrient utilization of pigs to xylanase, experimental diet formulation method for energy (FME), and DIM. In Exp. 1, a total of 448 male broiler chickens were used in a randomized complete block design with BW as a blocking factor. Seven dietary treatments were prepared in a 3 × 2 + 1 factorial arrangement with inclusion of sand, diatomaceous earth (DAE), or wheat bran (WB) as FME and without or with xylanase (26,400 unit/kg of diet) plus positive control, which contained sufficient energy content for animals. Each of Cr_2O_3 and

TiO_2 were incorporated at 5 g/kg in diets. In Exp. 2, twenty-one barrows (initial BW = 33.0 ± 0.3 kg), fitted with simple T-cannulas at the distal ileum, were used in a triplicate 7 × 2 incomplete Latin Square design with 7 dietary treatments, which were prepared by the same arrangement as in broilers. In Exp. 1, the growth performance of birds was not affected by xylanase, but was affected by the choice of FME. There were interactions ($P < 0.05$) between xylanase and FME for AID of CP, His, Met, Thr, and Trp. In Exp. 2, there were interactions ($P < 0.05$) between xylanase and FME for AID of dry matter, GE, Arg, and Lys. The DIM type had no effect on responses in pigs. In conclusion, the efficacy of xylanase on ileal energy and AA digestibility depends on the choice of FME in broilers and pigs, and DIM affects ileal digestibility in broilers.

In summary, the AID of GE or N was more influenced by the DIM type compared with ATTD, and the three DIM moved synchronously in diets irrespective of TP. The AID of GE and N, the ATTD of GE, and the recovery of Cr_2O_3 or TiO_2 were affected by DIM type, but not DIM level. However, the choice of DIM had no effect on the determination of BEL, AID, and SID of CP and AA, and additivity of AID and SID of CP and most indispensable AA in mixed diets. In addition, more accurate prediction of ileal digestibility of AA was achieved using SID rather than AID in mixed diets containing wheat, CM, MBM, and DDGS. Finally, the efficacy of xylanase on ileal energy and AA digestibility depends on the choice of FME in broilers and pigs, and DIM affects ileal digestibility in broilers.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Dietary formulations of feed fed to animals is made by combining and thoroughly mixing several feed ingredients to meet the requirements of animals for all nutrients. However, the efficiency of utilization of nutrient from different ingredients are different, and therefore it is important to estimate the absorption of nutrient from all the feed ingredients contained in the diet (NRC, 2012). One of the practical ways of assessing the absorbed quantities of nutrient and energy by animals is to determine digestibility (Stein and Bohlke, 2007). Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrient in feedstuffs are determined for pigs, but AID and apparent total tract utilization (ATTU) are determined for broiler chickens due to the voiding of feces and urine together in the excreta of birds. Determination of either ileal digestibility or total tract digestibility of amino acids (AA) and energy is affected by intestinal microbes as well as the absorption site of the nutrient of interest. To avoid the interference of microbiota on AA composition in ceca of broiler chicken and large intestine of pigs, ileal digestibility is usually determined for AA. For energy, on the other hand, total tract utilization is determined acknowledging the contribution of microbiota in hindgut.

To determine ileal digestibility of AA and total tract utilization of energy in pigs and broiler chickens, either the total collection method or index method could be used (Kong and Adeola, 2014). Compared to the total collection method, the index method is less laborious and therefore it has been widely used in studies conducted in pigs and broiler chickens. To apply the index method, digestibility index marker (DIM), which should be nonabsorbale, nonessential, nontoxic, indigestible, easily chemically analyzed, regularly and completely voided in feces, and uniformly mixed with samples and digesta, is required to be included in the diets of interest (Adeola, 2001).

The assumptions about DIM should be held under different circumstances, however, the violations such as low recovery of DIM has been reported in both pigs and chickens (Peddie et al., 1982; Jagger et al., 1992; Kavanagh et al., 2001). Therefore, the objective of the studies in this dissertation was to investigate the relationship between DIM and dietary characteristics including dietary fiber type, dietary protein sources, and inclusion of xylanase in the determination of nitrogen and energy utilization for pigs and broiler chickens.

1.2 Dietary fiber

Dietary fiber, which is also known as non-starch polysaccharide (NSP), is defined as carbohydrates that cannot be hydrolyzed by digestive enzymes in the small intestine, but some could be fermented by the intestinal microflora (De Vries, 2004). Due to the low available energy in fiber, high inclusion levels of fiber-rich feedstuffs are not recommended, especially in young animals with incompletely developed digestive systems. However, with the wide implementation of carbohydrases and constant pursuit of low cost diets, the usage of fiber-rich feed ingredients is increasing. Dietary fiber, consisting of cellulose, hemicellulose, pectins, fructans, glucomannans, galactomannans, mucilages, β-glucans and gums, is categorized in various ways (Grieshop et al., 2001). According to the function of NSP in plants, dietary fiber could be divided into cell wall components and non-cell wall components (NRC, 2012). On the other hand, according to its properties in aqueous solutions, dietary fiber is classified as soluble fiber and insoluble fiber, which have different physiological impact on nutrient utilization in animals.

1.2.1 Soluble fiber vs. insoluble fiber

Soluble fiber has high water-holding capacity and produces a gel-like substance in aqueous solution (Wursch and Pi-Sunyer, 1997). As a result, the soluble dietary fiber would decrease the

rate of passage of digesta in the gastrointestinal tract as well as be readily fermented by intestinal bacteria. Arabinoxylans from wheat and rye, and β-glucans which is prevalent in barley and oat are water-soluble fibers and are highly viscous in solutions (Friesen et al., 1992). Insoluble fiber does not dissolve in water and acts as bulking agents which speeds up intestinal transit time (Wursch and Pi-Sunyer, 1997). As a part of the cell wall in plants, dietary fiber might shield nutrient from contact with digestive enzymes or physically interfere with the process of digestion and absorption of nutrient (Adeola and Cowieson, 2011).

1.2.2 Dietary fiber effects on AA and energy utilization

Arabinoxylans and β-glucans have viscosity-inducing anti-nutritional effects, which makes nutrient inaccessible to digestive enzymes and consequently decreases the absorption of nutrient. To alleviate the adverse effect of arabinoxylans or β-glucans, exogenous xylanase or β-glucanase are usually added to diets. Exogenous xylanase could specifically cleave and hydrolyze the xylose backbone of arabinoxylans, and consequently reduces the viscosity of ileal digesta induced by arabinoxylans (Bedford and Schulze, 1998; Adeola and Bedford, 2004; Svihus, 2010). It has been reported that inclusion of xylanase in the diet improved the AID of AA and growth performance in growing pigs (Barrera et al., 2004). It has been summarized that the improvement of AID of AA from adding exogenous xylanase is higher in the feed ingredients with high concentrations of soluble fiber such as rye or barley (Cowieson and Bedford, 2009). Similarly, exogenous β-glucanase could also reduce the viscosity of ileal digesta by breaking down β-glucans (Barletta, 2010).

Insoluble fiber also has adverse effects on AID of AA by increasing the passage rate of digesta in the gastrointestinal tract and as a result of decreasing the contact time between pancreatic enzymes and nutrient. Furthermore, it has been reported that high dietary NSP content increased

intestinal epithelial cell turnover rate (Jin et al., 1994), which would affect specific endogenous losses of AA and decrease the efficiency of nitrogen utilization.

Like AA, the efficiency of energy utilization in small intestine is decreased in diets with high fiber concentration. However, some NSP could be readily fermented by intestinal bacteria mainly located in the hindgut, and produce a mixture of volatile fatty acids. Among the produced short-chain fatty acids, acetic, propionic, and butyric acids are the main components and could be rapidly absorbed as energy sources, although the efficiency of this process is relative low (Grieshop et al., 2001).

1.3 Amino acids

Amino acids supplied by crude protein (CP) play critical roles in animal nutrition because they are essential nutrient used by the body are the most expensive nutrient in the diets (NRC, 2012). Both relative bioavailability of AA and ileal digestibility of AA have been used to indicate the amount of AA that are available to animals (Lewis and Bayley, 1995). Slope-ratio assay used to determine relative bioavailability of AA is a time-consuming and expensive process, and therefore it is not a practical way to determine AA availability in feed ingredients and diets for animals (Kong and Adeola, 2014). Ileal digestibility assay is comparatively less expensive and more practical, as a result it has been widely used to determine the availability of AA. Depending on whether ileal digestibility is corrected for endogenous losses, AID, standardized ileal digestibility (SID), and true ileal digestibility (TID) of AA could be determined.

1.3.1 Ileal Digestibility of Amino Acids

Apparent ileal digestibility of AA is generally determined to avoid the modifying effects of bacterial metabolism on AA composition in the ceca of broiler chickens and the large intestine

of pigs, and therefore provides a more representative reflection of AA utilization (Sauer and Ozimek, 1986). The AID of AA is calculated using the following equations (Stein et al., 2005):

$$AID, \% = [(AA_{intake} - AA_{output})/AA_{intake}] \times 100;$$

where AA_{intake} and AA_{output} are the amount [kg dry matter (DM)] of AA in feed intake and ileal digesta output, respectively.

However, the digesta flow at the end of the ileum consists of not only nondigested dietary AA, but also endogenous losses of AA, which originate from pancreatic enzymes, mucin, bacterial protein, and sloughed epithelial cells (Gabert et al., 2001). Total ileal endogenous losses (TEL) of AA could be divided into basal ileal endogenous losses (BEL) of AA and specific endogenous losses of AA (Stein et al., 2007). Basal endogenous losses of AA represent the minimum quantities of AA inevitably lost by animals, which relates to the metabolic state of animals but is not influenced by dietary composition, however, specific endogenous losses of AA are induced by specific characteristics of feed ingredients (Stein et al., 2007). The content of BEL could be determined by feeding animals nitrogen-free diets, enzymically hydrolyzed casein diets, and low-protein casein diets, and the content of TEL of AA could be measured by the isotope dilution technique or homoarginine method (Gabert et al., 2001).

Standardized ileal digestibility is calculated by correcting AID for BEL, and therefore SID of AA is independent of dietary AA concentration and is more additive compared with AID of AA in mixed diets (Jansman et al., 2002). The more accurate prediction of ileal digestibility of AA has been achieved using SID rather than AID in corn-based mixed diets (Stein et al., 2005; Xue et al., 2014). True ileal digestibility of AA is calculated by correcting AID for TEL of AA. Due to the difficulty and high expense of measuring TEL of AA, TID of AA in most feed ingredients is not

available, which limits the application of TID of AA in diet formulation. The SID and TID of AA are calculated using the following equations (Stein et al., 2005):

$$SID, \% = AID + [(BEL/AA_{diet}) \times 100];$$

$$TID, \% = AID + [(TEL/AA_{diet}) \times 100];$$

where AA_{diet} is the AA concentration (g/kg DM) in test diet.

1.3.2 Total Collection Method for Determining Ileal Digestibility

Special surgical procedures are required for complete collection of ileal digesta from pigs. Ileo-rectal anastomosis (IRA) procedure involves the isolation of the cecum and large intestine from the gastrointestinal tract and then surgically attaching the ileum to rectum (Green et al., 1987), as a result ileal digesta could be entirely and directly collected from the anus. This IRA procedure functions well for the collection of digesta of pigs fed high fiber diets, but there are several concerns related to this method including interference to digestive processing with missing colon, animal welfare concerns, and uncontrollable digesta flow out of the anus of pigs (Gabert et al., 2001).

Re-entrant cannulation was introduced for the total collection of ileal digesta by Cunningham et al. (1963), where the flow of digesta was diverted outside of the pig, and then returned to either the ileum or the cecum. But this procedure is not widely used because the transection of the small intestine interferes the normal physiological state of animals and leads to cannula blockage in the pigs fed high fiber and large-particle-sized diets (Gabert et al., 2001).

Steered ileo-cecal valve (SICV) cannulation technique is a relative new collection technique that allows quantitative collection of ileal digesta from pigs via a valve-steering system (Mroz et al., 1996). The SICV approach could be used with many different types of diets (Gabert et al., 2001).

In chickens, instead of installing cannula, the ceca of chickens are removed to eliminate most of the effect of the microbes in the large intestine on nutrient utilization, which involves cecectomy (Parsons, 2002). By precisely feeding the cecectomized cockerel, the digestibility of AA could be determined with the total collection method in chickens.

1.3.3 Index Method for Determining Ileal Digestibility

Adding marker to a diet makes the determination of digestibility at one time point possible. In poultry, ileal digesta could be collected through a cannula, which is inserted into the terminal ileum, and the surgery technique was described in detail by Raharjo and Farrell (1984). A 30-mm long glass T-piece cannula is inserted to the desired site, which is about 7 to 10 mm anterior to the ileo-cecal junction of adult cockerels, and fixed by placing a rubber "O" ring on the base of the cannula to adhere to the abdominal muscle tissue. However, this procedure has not been commonly used due to the requirement of specific surgical expertise, blockage, and rejection of cannula (Parsons, 2002).

On the other hand, the slaughter method with a relatively simple procedure has been commonly used in poultry to collect ileal digesta with index method. The slaughter method of collecting ileal digesta from chickens or pigs involves euthanizing animals, dissecting the gastrointestinal tract, and sampling ileal digesta (Kong and Adeola, 2014). In pigs, however, the slaughter method is not as commonly used as in chickens for the determination of ileal digestibility, and the main reasons are the high expense to obtain enough replicates and the high pig-to-pig variation (Gabert et al., 2001). Instead, the simple T-cannula and post-valve T-caecum (PVTC) cannula procedures are generally used in pigs to determine ileal digestibility of nutrient.

Considering the normal physiological state of animals, allowance of spot-sampling, and absence of blockage problems with many types of diets, simple T-cannula method has become the

most popular procedure used in recent studies that require the collection of ileal digesta samples in pigs (Nyachoti et al., 1997; Gabert et al., 2001). Briefly, the simple T-cannula is inserted into the distal ileum of pigs, where approximately 6 cm anterior to the ileocecal junction, and the cannula is fixed at the last intercostal space of pigs (Dilger et al., 2004). Unlike simple T-cannula, PVTC procedure involves partial removal of cecum and replacement of it with PVTC cannula (van Leeuwen et al., 1991), which might influence the normal physiological status of the gastrointestinal tract, and as a result influence the measured digestibility of nutrient.

1.4 Energy

Energy is required for all the biological processes in animals, and therefore a precise estimation of the available energy value of feed ingredients and diets is necessary for optimizing animal production. Gross energy (GE) content of diet could be determined by calorimetry, however, the GE content of diet is not completely available to animals. Based on the GE value of diet, digestible energy (DE), metabolizable energy (ME), and net energy (NE) systems have been developed to evaluate the available energy value of the diet. Net energy is the closest estimation of the "true" energy value of feed ingredients because it considers the metabolic utilization of ME (Noblet, 2000), therefore it is more advanced compared with DE and ME systems, especially for high fiber or high protein feed ingredients. However, due to the high expense of determining NE and large variance of feed ingredients, NE system has not been widely applied in the evaluation of feed ingredients as well as diet formulation for pigs and chickens. Currently, DE and ME systems are widely used in the evaluation of feed ingredients in pigs, and the ME system is preferred in chickens due to the difficulty of separating feces from excreta.

1.4.1 Digestible and Metabolizable Energy Systems

In pigs, DE is calculated by subtracting the energy loss in feces from energy intake, and ME is determined by subtracting the energy loss in urine from DE. In chickens, ME is determined by subtracting energy loss in excreta from energy intake as follows (Kong and Adeola, 2014):

DE (kcal/kg DM) = $(GE_I - GE_F)/DMI$;

ME (kcal/kg DM) in pigs = $(GE_I - GE_F - GE_U)/DMI$;

ME (kcal/kg DM) in chickens = $(GE_I - GE_E)/DMI$;

where GE_I, GE_F, GE_U, and GE_E are the GE intake, and GE output in feces, urine, and excreta (kcal/d), respectively; DMI is DM intake (kg/d).

The values of ATTD and ATTU of GE equals the ratio between DE and GE and the ratio between ME and GE in test diets or ingredients. According to the collection method of outputs, ATTD and ATTU of GE could be determined by either the total collection method or index method.

1.4.2 Total Collection Method for Determining Energy Utilization

Total or quantitative collection of feces in pigs usually lasts 4 to 6 days after a 3-d to 7-d adaptation period, or a 5-d collection period after a 5-d adaptation period is suggested by Adeola (2001). In the procedural details, a small amount of color-marker [such as ferric oxide, chromic oxide (Cr_2O_3), carmine red, or indigo carmine] is added to 100 g of feed during the morning feeding on d 6 and d 11 to mark the initiation and end of the feces collection, respectively. The color-marked feed is provided to pigs first, and the remaining feed allotment for that mealtime is offered after pigs consume all the color-marked feed. Therefore, it is assumed that the feces voided and collected between the first and second appearances of the color-marked feces originate from the feed consumed between the administration of the two color-markers during the 5-d collection

period. To determine ME, urinary energy should be determined, however urine could not be marked with color-marker contained in diet. As a result, urine is generally collected from the morning on d 6 through the morning on d 11, which is consistent with the period when the color-marker is introduced.

In chickens, total collection of excreta consisting of feces and urine is usually conducted due to the difficulty of separating feces from excreta. The procedure of total collection of excreta in roosters is described by Sibbald (1976) in the determination of true ME in feedstuffs. In short, the bird is force-fed an accurate amount of feed after a 21-h starvation period, and then excreta are collected by placing a tray under cage for the upcoming 24-h period. Later, Parsons (2002) modified this process in the cockerels by extending both the fasting time and excreta collection period to 48 h, which is referred to the 'precision-fed cockerel assay'.

The ATTD and ATTU of GE in pigs and broiler chickens with the total collection method are calculated using the following equations (Adeola, 2001):

$$\text{ATTD of GE (\%)} = [(GE_d \times W_d) - (GE_f \times W_f)] / (GE_d \times W_d) \times 100;$$

$$\text{ATTU of GE (\%) in pigs} = [(GE_d \times W_d) - (GE_f \times W_f) - (GE_u \times W_u)] / (GE_d \times W_d)] \times 100;$$

$$\text{ATTU of GE (\%) in chickens} = [(GE_d \times W_d) - (GE_e \times W_e)] / (GE_d \times W_d)] \times 100;$$

where GE_d, GE_f, GE_u, and GE_e are the concentration (kcal/kg DM) of GE in diet, feces, urine, and excreta samples, respectively; W_d, W_f, W_u, and W_e are the weight (kg DM) of diet consumption, and output of feces, urine, and excreta samples during the collection period, respectively.

1.4.3 Index Method for Determining Energy Utilization

Similar to the total collection method, special surgery is not required in the determination of ATTD or ATTU of GE with the index method. Test diets with evenly distributed DIM is fed to animals, and representative fecal samples in pigs or excreta samples in broiler chickens are

collected through grab-sampling method. According to the description of grab-sampling feces in pigs by Moughan et al. (1991), approximately 20 g fecal samples are collected between 0830 and 1630h from collection trays daily, and then weighed and stored at –20°C. After the 6-d fecal collection period, all the feces sampled are bulked to form a composite sample for further chemical analysis.

The ATTD and ATTU of GE in pigs and broiler chickens with the index method are calculated using the following equations (Adedokun and Adeola, 2005):

$$\text{ATTD, \%} = [1 - (GE_f/GE_d) \times (DIM_d/DIM_f)] \times 100;$$

$$\text{ATTU, \%} = [1 - (GE_e/GE_d) \times (DIM_d/DIM_e)] \times 100;$$

where GE_d, GE_f, and GE_e are the concentration (kcal/kg DM) of GE in diet, feces, and excreta samples, respectively; DIM_d, DIM_f, and DIM_e are the DIM concentration (g/kg DM) of diet, feces, and excreta samples, respectively.

Comparison between the total collection method and the index method were conducted in the determination of ATTD of GE in pigs (Table 1-1). As shown in the table, DIM type and characteristics of basal diet might influence the measurement of energy utilization and gave inconsistent results. However, due to the rapid and relatively reliable alternative to the total collection method (Kavanagh et al., 2001), the index method with grab-sampling procedure is widely used in both academic and industry experiments.

1.5 Digestibility Index Markers Used in Non-Ruminant Animals

Inclusion of DIM as an index to determine digestibility avoids the requirement for quantitative records of feed intake and feces output (Adeola, 2001), and therefore the index method has been widely used in the determination of digestibility studies in non-ruminant animals. The characteristics that a qualified DIM should have include: completely inert in the gastrointestinal

tract, completely and regularly excreted, and uniformly mixed with the digesta or fecal materials (Kong and Adeola, 2014), which could be expressed in equation:

$$DIM_{intake} \times W_{intake} = DIM_{output} \times W_{output}$$

where DIM_{intake} and DIM_{output} are the DIM concentrations (mg/kg DM) in feed intake and outputs including ileal digesta, feces, or excreta samples, respectively; W_{intake} and W_{output} are the total weight (kg DM) of feed consumption and outputs including ileal digesta, feces, or excreta samples, respectively.

1.5.1 Categories of Digestibility Index Markers

Acid insoluble ash (AIA), Cr_2O_3, and TiO_2 have been the most common DIM used in both pigs and broiler chickens to determine ileal and total tract digestibility (McCarthy et al., 1974; Jagger et al., 1992; Sales and Janssens, 2003; Olukosi et al., 2012). In addition to these three DIM, other DIM including dysprosium chloride, lanthanum, samarium, and ytterbium chloride have been used in studies as summarized by Gabert et al. (2001). The specific physical and chemical attributes of each DIM, however, might have various effects on the determination of the nutrient and energy digestibility in animals.

1.5.1.1 Acid Insoluble Ash

Acid insoluble ash, also known as 4N-HCl insoluble ashes (McCarthy et al., 1974), consists of mineral components that are not digested in 4 N hydrochloric acid solution. The earliest usage of AIA as DIM was reported in 1874 (Sales and Janssens, 2003), due to the natural existence of it in feedstuffs, and the relatively simple, safe, and inexpensive chemical analysis, AIA has become one of the most popular DIM used in the determination of nutrient digestibility in pigs and broiler chickens.

The AIA concentration varies a lot in feed ingredients. The concentrations of AIA are less than 2 g/kg DM in corn, soybean meal, and cottonseed meal, however its concentrations are greater than 21 g/kg DM in fish meal and various kinds of hay (Sales and Janssens, 2003). McCarthy et al. (1974) reported that the AIA contained in feed for all growth phases of pigs varies from 6 to 8 g/kg, which was enough to obtain satasifatory results of total tract digestibility. Because the measurement method of AIA content is gravimetric determination, therefore the higher the quantity of AIA included in samples, the more reliable the results obtained. Therefore, extra compounds, mainly celite, but also including sand, acid-washed sand, volcanic ash, silica, and bentonite are always included in diets to contribute as dietary AIA components, as summarized by Sales and Janssens (2003).

1.5.1.2 Chromic Oxide

Chromic oxide is a green inorganic compound containing trivalent Cr with a molecular weight of 152.02 g/mol and x-ray density of 5.22 g/cm^3 (Udy, 1956b). Due to the color of Cr_2O_3, it is widely used as green pigment, and known as green oxide of Cr, anadonis green, chrome ocher, and leaf green, etc. Chromic oxide is insoluble in water, alcohol, and acetone, but is soluble in hot 70% perchloric acid, which could oxidize it to soluble Cr trioxide (Udy, 1956b).

The earliest report about utilizing Cr_2O_3 as DIM in the determination of digestibility in non-ruminant animals was in the 1950's (Moore, 1957) and it is still one of the most common DIM used in animal nutrition today, although several concerns related to it have arisen. One of the concerns about including Cr_2O_3 in diets as DIM is potential environmental pollution (Förstner, 1981) and potential carcinogenic activity of hexavalent Cr (Wetterhahn and Hamilton, 1989), which is formed from trivalent Cr during the chemical digestion process (Fenton and Fenton, 1979).

1.5.1.3 Titanium Dioxide

Titanium dioxide (TiO_2) containing tetravalent Ti is a white and almost tasteless powder with the density of 4.3 g/cm^3 (Peddie et al., 1982). Since the commercial production of TiO_2 in the early twentieth century, TiO_2 has been widely used as pigment in white paints, and also has been included in products including sunscreen and toothpaste (Braun et al., 1992; Pfaff and Reynders, 1999; Chen and Mao, 2007). The earliest research about using TiO_2 as DIM to determine nutrient utilization was reported in a rat study conducted in the late 1950's (Njaa, 1961).

1.5.1.4 Comparison among Digestibility Index Markers

Previous studies have shown that the choice of DIM might affect the determination of AID and ATTD of nutrient with the index method. It has been reported that TiO_2 gave greater ileal digestibility of nutrient in broilers and pigs installed with either a simple T-cannula or PVTC cannula compared with Cr_2O_3 (Jagger et al., 1992; Yin et al., 2000b; Olukosi et al., 2012; Favero et al., 2014), however, Köhler et al. (1990) reported completely opposite results, where Cr_2O_3 produced greater AID of nutrient than TiO_2.

Furthermore, van Leeuwen et al. (1996) reported that greater AID of CP was determined by Cr_2O_3 compared with AIA in wheat gluten bran diet, but similar AID of CP was determined by Cr_2O_3 and AIA in soybean meal diet. On the other hand, greater ATTD determined with AIA than Cr_2O_3 in pigs was reported by McCarthy et al. (1974), however, similar ATTD values determined by AIA and Cr_2O_3 was reported by Kavanagh et al. (2001). There is still disagreement among researchers about the possible reasons for these inconsistent results from the comparisons among DIM in the determination of nutrient utilization, which might be related to the discordant recovery of DIM as discussed below.

1.5.2 Recovery of Digestibility Index Markers

The recovery of DIM in ileal digesta, feces, and excreta samples are calculated according to the following equations:

Recovery of DIM in digesta, % = $(DIM_{digesta} \times W_{digesta}) / (DIM_{diet} \times W_{diet}) \times 100$;

Recovery of DIM in feces, % = $(DIM_{feces} \times W_{feces}) / (DIM_{diet} \times W_{diet}) \times 100$;

Recovery of DIM in excreta, % = $(DIM_{excreta} \times W_{excreta}) / (DIM_{diet} \times W_{diet}) \times 100$;

where DIM_{diet}, $DIM_{digesta}$, DIM_{feces}, and $DIM_{excreta}$ are the DIM concentration (mg/kg DM) of the diet, ileal digesta, feces and excreta samples, respectively; W_{diet}, $W_{digesta}$, W_{feces}, and $W_{excreta}$ are the weight (kg DM) of diet consumption, and output of ileal digesta, feces, and excreta samples during collection period, respectively.

The recovery of DIM in total tract output and ileal digesta in pigs and chickens are summarized in Table 1-2. In pigs, the recovery of AIA, Cr_2O_3, and TiO_2 in feces varied from 97% to 183% (Moughan et al., 1991; Bakker and Jongbloed, 1994; Kavanagh et al., 2001), 74.6% to 96% (Moore, 1957; Moughan et al., 1991; Jagger et al., 1992; Kavanagh et al., 2001), and 92.3% to 98.3% (Jagger et al., 1992; Kavanagh et al., 2001), respectively. In laying hens, the recovery of TiO_2 in excreta from pelleted diets was reported with a relative high value of 97.5% (Peddie et al., 1982).

The recovery of Cr_2O_3 and TiO_2 could also be determined in ileal digesta of pigs which allow quantitative collection of ileal digesta samples. The recovery of Cr_2O_3 in ileal digesta of pigs installed with re-entrant cannula was reported in the range of 82.3% to 93.6% (Köhler et al., 1990). It has been reported that the recovery of Cr_2O_3 in ileal digesta varied between 84.3% to 88.7% in pigs subjected to IRA procedure, which was less than the recovery of TiO_2 with the value between

95.2% to 101.1%, and the possible reason for the different recovery results might be related to the interference from dietary fiber (Yin et al., 2000b).

1.5.3 Reasons for Low Digestibility Index Markers Recovery

With the assumption that DIM should be completely and regularly excreted (Kong and Adeola, 2014), therefore, the recovery of DIM should be 100%. The recovery of DIM, however, largely varied from 100% and the possible explanations are numerous. Based on the calculation formula of recovery of DIM mentioned earlier, lower recovery of DIM might be caused by either underestimation of DIM in outputs or overestimation of DIM in feed intake.

1.5.3.1 Error in Measurement of Digestibility Index Marker Concentration

Digestibility index marker might interact with host animals in either biochemical or physical pathways. Greater phosphofructokinase activity and Cr concentration in tissues were observed in tilapia fed diets including greater levels of Cr_2O_3 (Shiau and Liang, 1995), and this result violated the assumption about DIM, that it should be completely inert in the gastrointestinal tract. Similarly, it has been reported that the nanoparticles of TiO_2 ingested by rainbow trout accumulated in some tissues and decreased the activity of Na-K-ATPase in brain, although it was neither growth-inhibiting nor lethal (Ramsden et al., 2009). Biochemical interactions between DIM and animal tissues were observed in aquatic animals, and it is reasonable to speculate that similar effects involving Cr or Ti might happen with pigs and broiler chickens.

Furthermore, there is evidence of Cr_2O_3 in the gastric gland region of the stomach and the crypts of the epithelium along the small intestine in pigs fed diets containing 0.5 g/kg Cr_2O_3 (Mroz et al., 1996). Either biochemical or physical interactions between DIM and animals might cause the loss of DIM in the animal body and as a result, decrease the recovery of DIM in outputs.

1.5.3.2 Error in Measurement of Sample Weight

In the calculation formula of recovery of DIM, the weight of feed consumption is calculated by subtracting the weight of wasted diet from total amount of feed supply, which has been calculated according to the BW of animals and therefore is a fixed number. However, it is challenging to completely collect all the wasted diet in pig experiment, therefore it leads to the overestimation of the weight of feed consumption and finally the underestimation of recovery of DIM in outputs. Kavanagh et al. (2001) reported that there was a small amount of Cr_2O_3 left on the sample bags, which belonged to the wasted feed and should be recorded. Furthermore, Peddie et al. (1982) reported relatively greater TiO_2 recovery in laying hens fed pelleted diets, which increases the accuracy of measuring the amount of wasted diet and therefore the weight of feed consumed.

On the other hand, a small amount of feces might stick to the metabolism crates used to house pigs (Moore, 1957) or be washed away by urine excreted from pigs during the fecal collection period, both situations are inevitable and might cause the loss and underestimation of the weight of output, and as a result decreases the recovery of DIM.

1.5.3.3 Cr Loss during Chemical Analysis

In the chemical analysis method of Cr described by Fenton and Fenton (1979), Cr_2O_3 reacts with perchloric acid to form Cr trioxide, which could be quantified by absorption spectrophotometry. However, Cr trioxide readily polymerizes with the loss of water to form dichromic acid and higher polymers, which could result in an underestimation of Cr concentration (Udy, 1956b).

Furthermore, Cr loss can occur through formation of volatile chlorine compound (Gorsuch, 1959) or bonding to glassware during the digestion process (Cary and Allaway, 1971). It is worth

noting that the loss of Cr during chemical analysis can occur in both diet and output samples, therefore it is difficult to predict its final effect on the recovery of Cr in output.

1.6 Factors Affecting Nutrient Digestibility Determined by Index Method

Except the choice of DIM, other factors including dietary characteristics, inclusion of exogenous enzymes, inclusion level of DIM in diet, and sampling procedure of digesta could also influence the determination of nutrient digestibility or utilization in pigs and broiler chickens.

1.6.1 Dietary Factor: Fiber and Enzyme

The interference of dietary fiber with DIM has been reported in the determination of ileal digestibility in pigs fitted with cannulas. It has been reported that different AID of DM in pigs fitted with simple T-cannula were determined by Cr and cobalt in corn-wheat-based diets rather than in fiber-rich and pectin-rich diets, which had larger standard error (Köhler et al., 1990). Furthermore, similar estimates of ATTD of nutrient were determined with the total collection method and Cr_2O_3 in pigs fed corn diets, however, the response criteria were consistently underestimated by Cr_2O_3 in pigs fed triticale diets (Adeola et al., 1986). On the other hand, the first appearance of Cr_2O_3 in the ileum of pigs was reported to be affected by dietary fiber (Owusu-Asiedu et al., 2006).

In broiler chickens, greater ATTU of DM and nitrogen-corrected ME was determined by TiO_2 in Rustic and Viscount wheat cultivars compared with Orpheus wheat cultivar, and the possible reason might relate to the amount of non-starch polysaccharide in various cultivars of wheat (Smeets et al., 2015). Furthermore, it has been reported that high viscosity digesta might cause uneven flow rates of Cr_2O_3 in broiler chickens (Scott and Boldaji, 1997).

Xylanase and phytase are the two exogenous enzymes that are commonly included in commercial feeds in swine and poultry industry (Adeola and Cowieson, 2011). By hydrolyzing dietary fiber, xylanase might interfere with the interaction between fiber and DIM (Yin et al., 2000a; Wang et al., 2017). In addition to hydrolyzing insoluble phytic acid in the diet, the inclusion of exogenous phytase might also affect the utilization of AA, Ca, and energy. The ileal digestibility of AA was notably improved in the studies where TiO_2 or AIA was used as DIM, but no evident effect was observed in the studies where Cr_2O_3 was used as DIM in most cases (Adeola and Cowieson, 2011). Similarly, the effect of inclusion of exogenous phytase on AID or SID of AA was reported to be dependent on the choice of Cr_2O_3 or TiO_2 as DIM when canola meal was used as the protein source in growing pigs (Favero et al., 2014).

1.6.2 Inclusion Level of Digestibility Index Markers in Diets

The inclusion level of DIM used in research conducted with non-ruminant animals mostly ranges from 1 to 10 g/kg, and 1, 3 or 5 g DIM has been commonly included per kg diet (Table 1-2). Based on the assumptions in the use of DIM, the nutrient digestibility values determined by different concentrations of DIM theoretically should be accordant. However, inconsistent results about the effect of inclusion level of DIM on nutrient digestibility have been reported in pigs, broiler chickens, and aquaculture.

To obtain accurate and repeatable results using AIA as DIM, 5 to 10 g/kg concentration is the recommended level in diets fed to pigs (Scott and Boldaji, 1997). Olukosi et al. (2012) reported that the AID of His and Trp in corn-soybean meal-based diets were greater at 3 g/kg DIM concentration than 5 g/kg in broilers, either using Cr_2O_3 or TiO_2 as DIM. In addition, greater digestibility was determined in the diets containing 5 g/kg Cr_2O_3 compared with the diet containing 20 g/kg Cr_2O_3 in tilapia (Shiau and Liang, 1995). In rainbow trout, however, greater Cr_2O_3

concentration at 20 g/kg had greater digestibility than at 5 or 10 g/kg Cr_2O_3 (Tacon and Rodrigues, 1984). Inconsistent effects of dietary DIM concentrations on the utilization of nutrient might be explained by the biochemical interference from DIM on host animals (Shiau and Liang, 1995; Ramsden et al., 2009).

Another concern of feeding animals diets containing high concentrations of DIM is related to feed consumption. Jagger et al., (1992) reported that the diets containing 5 g/kg Cr_2O_3 and TiO_2 were consumed at a slower rate compared with the diets containing 1 g/kg Cr_2O_3 and TiO_2. Furthermore, including high levels of DIM in diets would increase the feed cost. However, low concentrations of DIM in test diets is also not suggested because low DIM concentrations would increase the error of measurement in the chemical analysis of DIM concentration in samples. Therefore, more studies are required for determining the optimum level of DIM that should be added to diets.

1.6.3 Sample Collection Procedure

The standard procedure of sampling ileal digesta from pigs installed with T-cannula is collection of ileal digesta between two meals for two consecutive days to obtain representative samples. Jagger et al. (1992) reported that there was no statistical difference in the determination of AID of AA between collection days in pigs. However, the AID of DM determined on the first sample collection day was less than the values determined on the subsequent days as reported by Hill et al. (1996) in dogs. Furthermore, 6-h ileal digesta sample collection periods starting 4 to 6 h after feeding provided similar concentrations of Cr, flow of DM, BEL of AA, and SID of AA compared to a 12-h sample collection period in pigs fed various diets (Kim et al., 2016, 2017). Thus, further research is required to investigate the effect of ileal digesta collection procedure including collection day and time period on nutrient utilization in pigs.

1.7 Chemical Analysis Methods

The accuracy of quantitative analysis of DIM in feed and output samples is critical to the determination of nutrient utilization in animals. The recovery of Cr_2O_3 in diet and output samples varied between 97.2% and 104.3% (Saha and Gilbreath, 1991), and the recovery of TiO_2 in diet and output samples is between 98.7% to 99.99% (Peddie et al., 1982; Short et al., 1996).

1.7.1 Measurement of AIA

Due to the gravimetric method of AIA determination, relatively large amounts of sample is required to accurately determine the AIA contents in samples, which might constrain the use of AIA as DIM when the amount of collected sample is limited. The chemical reagents and analytical methods involved in the measurement of AIA are similar (Sales and Janssens, 2003).

Take the procedure described by McCarthy et al. (1974) as an example, approximately 10 g diet or feces is boiled in 100 mL 4 N hydrochloric acid for 30 min, and then filtered through ashless filter paper. The residue left on filter paper are washed with boiling water until the pH was neutral, and then placed in a forced-air oven until dry and ashed at 650°C for a minimum of 6 h. The modifications to this method including concentration of hydrochloric acid, boiling time, and ashing conditions were described by Sales and Janssens (2003).

1.7.2 Measurement of Cr

The atomic weight of Cr is 52.01 (Udy, 1956a) and it accounts for 68.4% molar mass of Cr_2O_3. The chemical analysis method of Cr went through a series of modifications including digestion procedure (Kimura and Miller, 1957; Czarnocki et al., 1961; Fenton and Fenton, 1979) and determination method (Green, 1975; Saha and Gilbreath, 1991) as shown in Table 1-3.

Before digesting Cr_2O_3, the organic matter in samples is removed by either reacting with nitric acid under heating condition or ashing in a muffle furnace directly. The Cr_2O_3 could be

dissolved and oxidized by purified perchloric acid to soluble Cr trioxide (Udy, 1956b). Purified perchloric acid (Kimura and Miller, 1957; Saha and Gilbreath, 1991) or a mixture of perchloric acid and other reagents, such as sulfuric acid, sodium molybdate, or silver nitrate (Czarnocki et al., 1961; Cary and Allaway, 1971; Fenton and Fenton, 1979) are added after the removal of organic matter. Chromium trioxide readily polymerizes with the loss of water to form dichromic acid and higher polymers (Udy, 1956b), which might underestimate the determination of Cr in samples.

In the method described by Williams et al. (1962), instead of hydrochloric acid, phosphoric acid-manganese sulfate solution and potassium bromate solution, which readily oxidizes Cr_2O_3 (Udy, 1956b), are added into the solution and heated until the formation of purple color. Before pouring the digested liquid into volumetric flask, calcium chloride is added to reduce the interference of other elements. The digested Cr solution could be either centrifuged or left to stand overnight to precipitate the impurities and be ready for the determination of Cr.

The amount of Cr in digested samples could be measured by either colorimetry or atomic absorption spectrophotometry. When Cr_2O_3 reacts with perchloric acid, a yellowish color of Cr trioxide is formed (Fenton and Fenton, 1979), which could be determined by spectrophotometer at 420 to 440 nm. However, the stability of the colored liquid is low, and therefore the measurement of Cr should be conducted quickly. With atomic absorption spectrophotometry, it was reported that Ca, silicate, aluminum, and magnesium in aqueous solution interfered in the determination of Cr (Williams et al., 1962), and later Saha and Gilbreath (1991) indicated that this type of interference conceivably could happen in colorimetric determination of Cr.

1.7.3 Measurement of Ti

The atomic weight of Ti is 47.87 and it accounts for 60.0% of the molar mass of TiO_2. Compared with Cr, the methods of measuring Ti are relatively consistent among all the methods as described in Table 1-4. In the digestion process of Ti, feed or feces could either go through an ashing process right before boiling samples with sulfuric acid (Leone, 1973; Short et al., 1996), or be heated up directly at 420°C for a few hours without ashing samples (Njaa, 1961; Myers et al., 2004). Reaction catalysts, such as copper, Kjeldahl tablet containing 3.5 g of potassium sulfate and 0.4 g copper sulfate per tablet, or sodium sulfate could be included in solution to speed up the digestion process (Myers et al., 2004).

Hydrogen peroxide with the concentration of 30% is added into digested liquid to form the intense orange or yellow color of peroxytitanic acid, which could be determined by spectrophotometry at 408 or 410 nm wavelength (Short et al., 1996; Myers et al., 2004). Different from Cr, the colored compound formed with Ti is stable for at least 9 weeks (Myers et al., 2004).

1.7.4 Interference among Digestibility Index Markers

In the study that compared different types of DIM, more than one DIM might be included in each diet to eliminate the confounding effect from experimental animals. Dietary formulation containing both Cr_2O_3 and TiO_2 (Jagger et al., 1992; Yin et al., 2000b; Olukosi et al., 2012), both AIA and Cr_2O_3 (Moughan et al., 1991), or AIA, Cr_2O_3, and TiO_2 (Kavangh et al., 2001) were reported in previous studies.

In the diets containing both Cr_2O_3 and TiO_2 as DIM, the determination of Ti by colorimetry was not affected by the dissolved Cr present in digestion liquid (Myers et al., 2004; Olukosi et al., 2012). However, TiO_2 could not be dissolved in the digestion process of Cr_2O_3, which might interfere with the determination of Cr by spectrophotometry. It has been reported that the oxidation

of Cr_2O_3 to Cr trioxide occurs more rapidly when Cr_2O_3 is heated with oxidizing agents including alkali nitrates, chlorates, dioxides than oxygen (Udy, 1956b). On the other hand, the inclusion of Cr_2O_3 or TiO_2 in the diet would increase the content of AIA in samples above the expected value because both Cr_2O_3 and TiO_2 could not be digested in hydrochloric acid, and therefore are determined as part of the AIA (Kavanagh et al., 2001).

1.8 Summary

The current literature review briefly introduced the methodology of the determination of ileal and total tract utilization of AA and energy in both pigs and broiler chickens. Dietary fiber has specific characteristics and its effect on AA and energy utilization in animals was reviewed. Then total collection method and index method in the determination of ileal digestibility of AA and total tract utilization of energy were compared. The three DIM commonly used in non-ruminant animals including AIA, Cr_2O_3, and TiO_2 were briefly introduced and compared with one another. After the introduction, the formula used to calculate the recovery of DIM was introduced, and then the literature values of the recovery of various DIM were discussed and compared with each other. The possible reasons for less than complete recovery of DIM were presented from different aspects. Then the factors including dietary characteristics, DIM level, and sampling procedure, which might interfere with the determination of nutrient utilization determined by index method were discussed. Finally, the developmental history and modifications of the chemical analytical methods for AIA, Cr, and Ti were presented.

1.9 Objective

The objective of this study was to investigate the methodology of the determination of nutrient utilization in growing pigs and broiler chickens. At first, the relationship between DIM

type and dietary fiber type in the determination of energy and nitrogen digestibility and DIM recovery was investigated in growing pigs. Then the effect of dietary fiber type, ileal digesta collection day, and time period on concentration patterns of DIM was investigated in growing pigs. Furthermore, the effect of DIM type, DIM level, and dietary fiber type on AID and ATTD of nutrient and energy, and recovery of DIM was determined in growing pigs. In addition, the additivity of AID and SID of CP and AA in mixed diets containing wheat and multiple protein sources with both Cr_2O_3 and TiO_2 as DIM was investigated. Finally, the effect of xylanase, diet formulation method for energy, and choice of DIM on nutrient and energy utilization for broiler chickens and pigs were investigated.

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Table 1-1. Comparison between index method and total collection method (TC) in the determination of apparent total tract digestibility (ATTD) of energy in pigs[1]

DIM				
Type	Level (g/kg)	Animals	Basal diet	Compared with index method, TC determined
AIA	0	Pig	Wheat-barley	similar ATTD of GE[2]
AIA	< 1	Pig	Barley	similar ATTD of GE[3]
AIA	5	Pig	Wheat-oat-barley	greater ATTD of GE[4]
Cr_2O_3	1	Pig	Wheat-barley	similar ATTD of GE[2]
Cr_2O_3	3	Pig	Corn	similar ATTD of GE[5]
Cr_2O_3	3	Pig	Triticale	greater ATTD of GE[5]
Cr_2O_3	-	Pig	Barley	greater ATTD of GE[3]
Cr_2O_3	5	Pig	Wheat-oat-barley	greater ATTD of GE[4]
Cr_2O_3	1 or 5	Pig	Barley-wheat-SBM	greater ATTD of GE[6]
TiO_2	1 or 5	Pig	Barley-wheat-SBM	greater ATTD of GE[6]
TiO_2	1	Pig	Wheat-barley	greater ATTD of GE[2]

[1]DIM = digestibility index marker; AIA = acid insoluble ash; Cr_2O_3 = chromic oxide; GE = gross energy; TiO_2 = titanium dioxide; SBM =soybean meal.

[2](Kavanagh et al., 2001)

[3](Moughan et al., 1991)

[4](McCarthy et al., 1974)

[5](Adeola et al., 1986)

[6](Jagger et al., 1992)

Table 1-2. Recovery (%) of digestibility index marker (DIM) in ileal digesta and total tract output determined by total collection method[1]

DIM					
Type	Level g/kg	Animals	Location	Collection period (d)	Recovery
AIA	-	Pig	Total tract	3 or 10	97-183[2]
AIA	3.3	Pig	Total tract	6	98.2[3]
AIA	-	Pig	Total tract	5	99.9[4]
Cr_2O_3	1	Pig	Total tract	5	74.6[5]
Cr_2O_3	10	Pig	Total tract	4	78 to 86[6]
Cr_2O_3	5	Pig	Total tract	5	79.7[5]
Cr_2O_3	2.5	Pig	Total tract	6	85.3[3]
Cr_2O_3	1	Pig	Total tract	5	96.0[4]
TiO_2	1	Pig	Total tract	5	92.3[4]
TiO_2	5	Pig	Total tract	5	96.9[5]
TiO_2	1	Pig	Total tract	5	98.3[5]
TiO_2	2	Laying hen	Total tract	10	97.5[7]
Cr_2O_3	0.5	Pig	Ileal digesta with RC	-	82.3 to 93.6[8]
Cr_2O_3	-	Pig	Ileal digesta with IRA	-	84.3 to 88.7[9]
TiO_2	-	Pig	Ileal digesta with IRA	-	95.2 to 101.1[9]

[1]AIA = acid insoluble ash; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; RC = re-entrant cannulation; IRA = ileo-rectal anastomosis.

[2](Bakker and Jongbloed, 1994)

[3](Moughan et al., 1991)

[4](Kavangh et al., 2001)

[5](Jagger et al., 1992)

[6](Moore, 1957)

[7](Peddie et al., 1982)

[8](Köhler al., 1990)

[9](Yin et al., 2000b)

Table 1-3. Method to determine Cr in feed and output[1]

Ash	Nitric acid	Digestion mixture	Purification	Measurement	Reference
No	10 mL (sodium molybdate)	5 mL perchloric acid	Centrifuge	SPM at 440 nm	Kimura and Miller, 1957
No	10 mL (store overnight)	Perchloric acid, sulfuric acid, and sodium molybdate	Stand overnight	SPM at 430 nm	Czarnocki et al., 1961
No	10 mL	Perchloric acid, sulfuric acid, silver nitrate, and sodium sulfate	-	AAS	Cary and Allaway, 1971
No	5 mL	7 mL perchloric acid	-	AAS or SPM at 420 nm	Saha and Gilbreath, 1991
Yes	-	Phosphoric acid-manganese sulfate solution, and potassium bromate solution (add calcium chloride after the formation of purple color)	Stand overnight	AAS	Williams, 1962
Yes	-	Perchloric acid + sulfuric acid + sodium molybdate dihydrate	Centrifuge	SPM at 440 nm	Fenton and Fenton, 1979

[1]AAS = atomic absorption spectrophotometry; SPM = spectrophotometer.

Table 1-4. Method to determine Ti in feed and output[1]

Ash	Sulfuric acid	Catalyst	Temperature, °C	Hydrogen peroxide	Measurement	Reference
No	Concentrated	Copper	420	30%	SPM at 408 nm	Njaa, 1961
No	Concentrated	Kjeldahl tablet[2]	420	30%	SPM at 410 nm	Myers et al., 2004
Yes	Concentrated	Sodium sulfate	Boiling	30%	SPM at 408 nm	Leone, 1973
Yes	Dilute to 7.4 M	-	Boiling	30%	SPM at 410 nm	Short et al., 1996

[1]SPM = spectrophotometer.

[2]Each tablet contains 3.5 g of potassium sulfate and 0.4 g copper sulfate.

CHAPTER 2. COMBINATION OF DIGESTIBILITY INDEX MARKER AND FIBER AFFECT ENERGY AND NITROGEN DIGESTIBILITY IN GROWING PIGS

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2.1 Abstract

This study was conducted to investigate if (i) the apparent ileal digestibility (AID) of gross energy (GE) or nitrogen (N) was influenced by the type of digestibility index marker (DIM) and dietary fiber, and (ii) the apparent total tract digestibility (ATTD) of GE or N was influenced by the type of method [method i.e. total collection (TC) and DIM] and dietary fiber. Eighteen barrows fitted with a T-cannula at the end of the ileum were used in a 2-period randomized complete block design. Three corn-soybean meal-based diets were formulated with corn starch (CS), corn bran (CB) or oat bran (OB) at 100 g/kg. All 3 diets contained 3 DIM, which were chromic oxide (Cr_2O_3), titanium dioxide (TiO_2), and acid-insoluble ash (AIA). The ileal digesta were collected for 3 days, and the AID of GE and N were determined by measuring Cr_2O_3, TiO_2 or AIA. The feces were collected by using TC method, and the ATTD of GE and N were determined by using Cr_2O_3, TiO_2, AIA, or TC method. There were interactions between diet and DIM ($P < 0.001$) for AID and DIM recovery ($P < 0.001$), and between diet and method for ATTD ($P < 0.001$). The DIM had similar effect on AID of GE and N within each diet, but different effects among the 3 diets. For CS and CB, the greatest AID of GE or N was determined by TiO_2, while for OB, the greatest AID was determined by AIA. However, the ATTD of GE or N of CS and the ATTD of N of CB determined by the 3 DIM were not different. The greatest ATTD of GE of CB was determined by TiO_2 or AIA, while the greatest ATTD of N or GE of OB was determined by Cr_2O_3 or AIA. For all 3 diets,

the ATTD of GE and N determined by the TC method was greater ($P < 0.001$) than those determined by using DIM. The recovery of TiO_2 in feces of pigs fed the OB was 78.3%, which was the least among all the 3 diets ($P < 0.05$). In conclusion, the AID of GE or N was more influenced by the choice of DIM compared with ATTD, and the TiO_2 recovery of pigs fed OB was less than CS or CB.

Key words: digestibility index marker, energy digestibility, nitrogen digestibility, corn bran, oat bran

2.2 Introduction

Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of gross energy (GE) and nitrogen (N) are commonly used to evaluate digestibility of feed nutrient and energy. The most common method used to determine AID is to include digestibility index marker (DIM) in the diet, and spot-sample ileal digesta from pigs fitted with simple T-cannulas (Köhler et al., 1990). For the determination of ATTD, either the total collection (TC) method or the DIM method can be used (Kong and Adeola, 2014). Chromic oxide (Cr_2O_3) titanium dioxide (TiO_2), and acid-insoluble ash (AIA) are the most common DIM used in pigs to determine AID and ATTD (McCarthy et al., 1974; Jagger et al., 1992). Total collection method determined similar energy digestibility with Cr_2O_3 in corn or with TiO_2 in wheat based complete diet, while greater values than using Cr_2O_3 in triticale diet (Adeola et al., 1986; Thompson and Wiseman, 1998).

Previous studies have indicated that the choice of DIM could affect the determination of AID and ATTD of nutrient (Jagger et al., 1992; Yin et al., 2000; Kavanagh et al., 2001; Favero et al., 2014). What's more, the effects of dietary fiber on nutrient digestibility have been investigated in pigs (Köhler et al., 1990) and broiler chickens (Smeets et al., 2015), however, the interaction between DIM and dietary fiber types have not been reported. Oat bran (OB) is rich in water-soluble

fiber and low in cellulose and lignin (Jacobs et al., 1983), but the major components of corn bran (CB) are cellulose and hemicellulose which are nearly completely insoluble in water (Rose et al., 2010). The hypothesis of this study was that there is no interaction between diet and DIM for AID, and method (i.e. TC and DIM) for ATTD. Therefore, the objective of the current study was to determine the effect of diet and DIM (or method) on AID (or ATTD) of GE and N in growing pigs.

2.3 Materials and Methods

All animal procedures used in this study were approved by the Purdue Animal Care and Use Committee.

Three corn-soybean meal-based diets containing 100 g/kg corn starch (CS), CB or OB, and identical in other components (Table 2-1) were prepared. All 3 diets contained 3 DIM, which were 5 g/kg Cr_2O_3, 5 g/kg TiO_2, and 20 g/kg AIA (Perma-Guard, Inc., Bountiful, UT).

Eighteen Hampshire × Duroc × Yorkshire × Landrace barrows were surgically fitted with a simple T-cannula at the distal ileum as described by Dilger et al. (2004). All pigs were housed individually in stainless steel metabolism crates (1.22 × 1.22 m) during the entire experimental period. Metabolism crates were equipped with a feeder and a nipple drinker and placed in a room with a 24-h lighting program. In the first period, 18 pigs were grouped into 6 blocks based on BW (24.2 ± 0.3 kg) and were randomly allotted to 3 diets. In the second period, the same 18 pigs were grouped into 6 new blocks based on BW (26.9 ± 0.5 kg) and went through the same procedure except that pigs consumed diets different from the first period, providing 12 observations per diet in total.

At the beginning of the experimental period, daily feed allowance was set as 4% of BW and pigs were fed at 08:00 and 16:00 h in 2 equal meals. Water was provided to the pigs via nipple

drinkers at 3 L/kg feed intake. Each period consisted of a 7-d adjustment period followed by a 3-d total fecal collection period and a 3-d ileal digesta collection period. Feces were collected using the TC method and collection was initiated and ended with the appearance of ferric oxide in feces as described by Akinmusire and Adeola (2009). The ileal digesta were collected every 3 h between 09:00 to 21:00 h with 4 time period (TP) where TP 1 = 09:00 to 12:00 h, TP 2 = 12:00 to 15:00 h, TP 3 = 15:00 to 18:00 h, and TP 4 = 18:00 to 21:00 h on ileal digesta collection days by attaching a Whirlpak® bag (NASCO, Fort Atkinson, WI) to the cannula with a rubber O-ring. The ileal digesta collection procedure used in this study was used to investigate the effects of TP and ileal collection day on DIM concentration Wang and Adeola (2017).

2.3.1 Chemical Analysis and Calculation

Ileal digesta from each pig at each TP within each ileal digesta collection day and feces samples for each pig within each period were pooled, subsampled, forced-air dried at 55°C to constant weight and ground to pass through a 0.5-mm screen before analyses. The dry matter (DM) contents of ileal and fecal samples were determined by drying at 105°C in a forced-air oven (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006) for 24 h. Chromium content was analyzed according to the method described by Saha and Gilbreath (1991) with modification of temperature and measured by spectrophotometer at 450 nm of absorption (Spectronic 21D; Milton Roy Co., Rochester, NY). Titanium content was analyzed as described by Myers et al. (2004). Acid-insoluble ash content was determined as described by McCarthy et al. (1974). Gross energy content was determined by isoperibol bomb calorimetry using a Parr 1261 calorimeter (PARR Instrument Co., Moline, IL). Nitrogen content was analyzed with the combustion method using a LECO Model TruMac® N analyzer (LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000). Acid detergent fiber was expressed inclusive of residual ash (method 973.18 (AD); AOAC,

2006) and neutral detergent fiber was assayed with a heat stable amylase and expressed inclusive of residual ash (Van Soest et al., 1991) and both were analyzed by the ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY).

The AID and ATTD of N were calculated using the following equations (Adedokun and Adeola, 2005):

AID of N = $[1 - (M_{diet}/M_{digesta}) \times (N_{digesta}/N_{diet})]$;

ATTD of N = $[1 - (M_{diet}/M_{feces}) \times (N_{feces}/N_{diet})]$,

where M_{diet}, $M_{digesta}$ and M_{feces} are the DIM concentration of the diet, ileal digesta and feces, respectively (g/kg of DM); N_{diet}, $N_{digesta}$ and N_{feces} are the N concentration of the diet, ileal digesta and feces, respectively (g/kg of DM). These equations were also used to calculate AID of GE and ATTD of GE with N replaced by GE (MJ/kg of DM). The value of $M_{digesta}$, $N_{digesta}$ or $GE_{digesta}$ were determined by pooling and taking an average of the DIM, N, or GE concentration of ileal digesta data for each pig within each period, separately.

2.3.2 Statistical Analysis

The AID, ATTD or DIM recovery data were analyzed as a two-way ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model used for AID and DIM recovery was:

$$Y_{ijklmn} = \mu + Diet_i + DIM_j + (Diet \times DIM)_{ij} + Block_k + Animal_l + (Animal \times Period)_{lm} + (Animal \times DIM)_{lj} + \varepsilon_{ijklmn}$$

where: Y_{ijklmn} = observation, μ = population mean, $Diet_i$ = diet effect (i = 1 to 3), DIM_j = digestibility index marker effect (j = 1 to 3), $(Diet \times DIM)_{ij}$ = interaction of Diet and DIM, $Block_k$ = block effect (k = 1 to 12), $Animal_l$ = animal effect (l = 1 to 18), $(Animal \times Period)_{lm}$ = interaction of Animal and Period (m = 1 to 2), $(Animal \times DIM)_{lj}$ = interaction of Animal and DIM, and ε_{ijklmn}

= residual error. In the model, Diet, DIM, and (Diet × DIM) were fixed effects; Animal, (Animal × Period), and (Animal × DIM) were random effects.

The model used for ATTD was:

$Y_{ijklmn} = \mu + Diet_i + Method_j + (Diet \times Method)_{ij} + Block_k + Animal_l + (Animal \times Period)_{lm} + (Animal \times Method)_{lj} + \varepsilon_{ijklmn}$

where: Y_{ijklmn} = observation, μ = population mean, $Diet_i$ = diet effect (i = 1 to 3), $Method_j$ = method effect (j = 1 to 4), $(Diet \times Method)_{ij}$ = interaction of Diet and Method, $Block_k$ = block effect (k = 1 to 12), $Animal_l$ = animal effect (l = 1 to 18), $(Animal \times Period)_{lm}$ = interaction of Animal and Period (m = 1 to 2), $(Animal \times Method)_{lj}$ = interaction of Animal and Method, and ε_{ijklmn} = residual error. In the model, Diet, Method, and (Diet × Method) were fixed effects; Animal, (Animal × Period), and (Animal × Method) were random effects. Least squares means were calculated and separated by the TDIFF option with Tukey's adjustment. Statistically significant difference was set at $P < 0.05$ and trend was set at $0.05 < P < 0.10$.

2.4 Results

As presented in Table 2-2, there were interactions between diet and DIM ($P < 0.001$) for AID of GE and N, and therefore the simple effect of the interaction between diet and DIM; and the main effects of diet and DIM are presented. Furthermore, standard error of difference (SED) of two means for diet × DIM appropriate for comparing DIM means within diet and SED for DIM × diet appropriate for comparing diet means within each DIM are listed in Table 2-2. The DIM comparisons within diet using appropriate SED of two means are discussed below. The DIM had a similar effect on AID of GE and N within each diet, but different effect among the 3 diets. For CS, the AID of GE or N determined by TiO_2 was greater than Cr_2O_3, which was greater than AIA. For CB, the TiO_2 determined a greater AID of GE or N than Cr_2O_3 and AIA. For OB, the AIA

determined the greatest AID of GE or N, although there was no difference between TiO_2 and Cr_2O_3.

There were interactions between diet and method for ATTD of GE and N ($P < 0.001$), and thus the simple effects of the interaction between diet and method; and the main effects of diet and method are shown in Table 2-3. Similar to AID, two SED values for the interaction between diet and method are listed. For all 3 diets, the ATTD of GE or N calculated using the TC method was greater ($P < 0.05$) than those calculated based on DIM (Table 2-3). Using the appropriate SED of two means for comparing method within diet, DIM had similar effect on ATTD of GE and N of CS and OB, but not true for CB. For CS, there was no difference of ATTD of GE or N determined by all the 3 DIM. For the ATTD of GE of CB, the TiO_2 determined a greater value than Cr_2O_3, while there was no difference for ATTD of N of CB. For OB, the ATTD of GE or N determined by AIA was greater than TiO_2, while there was no difference between Cr_2O_3 and TiO_2.

The interaction between diet and DIM on recovery of DIM in feces was significant ($P < 0.001$). Simple effects of the interaction between DIM and diet, and the main effects of DIM and diet; and two SED values for the interaction between DIM and diet are presented in Table 2-4. The diet comparisons within DIM using appropriate SED of two means are discussed below. The recovery of TiO_2 in feces of pigs fed the OB was 78.3%, which was less than that of the CS or CB ($P < 0.05$; Table 2-4). For Cr_2O_3 recovery, there was no statistical difference among the 3 diets, while the Cr_2O_3 recovery varied from 80.9% to 87.9%. For AIA recovery, there was no difference among the 3 diets, and it varied from 83.4% to 85.8%.

2.5 Discussion

In this study, the effects of DIM (or method) on AID (or ATTD) among the three diets were tested and we found DIM (or method) and diet affected AID (or ATTD) of GE and N, and

the TC method determined greater ATTD compared with using DIM which can be partly explained by the low recovery of DIM.

Interactions of diet and DIM for AID of N were observed in this study, which is consistent with Yin et al. (2000), where interaction between fiber level and method (DIM and post-valve ‘‘T’’ caecal cannulation method) was observed. In the current study, the choice of DIM affected the AID of GE or N within each diet, and had different pattern across the 3 diets. Greater value of AID was determined by TiO_2 compared with Cr_2O_3 (Olukosi et al., 2012), which agrees with the results with CS and CB in this study. What's more, Jagger et al. (1992) observed a similar result and stated that the low dietary Cr_2O_3 level had a greater standard error than TiO_2 and might be the reason. The greatest AID of GE and N of CS or CB were determined by TiO_2, while for OB, AIA determined the greatest AID of GE and N. The possible reason that accounts for the difference between OB and the other 2 diets is the greater interaction between TiO_2 and OB, and this can be indicated by the lower recovery of TiO_2 in feces of pigs fed OB. However, Köhler et al. (1990) reported that the AID of DM of both a pectin-rich diet and a crude-fiber-rich diet were not influenced by the choice of DIM which were Cr_2O_3 and Cobalt.

The ATTD of GE or N was less affected by the choice of DIM compared with AID, especially in CS. The possible reason for the different trend of choice of DIM on AID and ATTD of GE or N might be the depletion of nutrient, especially digestible cellulose (Shi and Noblet, 1993) in the hindgut caused by the fermentation, and therefore less interaction between DIM and diets was observed. The greatest ATTD of GE and N of OB was still determined by AIA or Cr_2O_3, while for CS or CB, TiO_2 or Cr_2O_3 determined the greatest ATTD of GE and N, and the possible reason might be similar with AID of OB mentioned above. And Kavanagh et al. (2001) reported

that the ATTD of GE of diets based on wheat and barley calculated by TiO_2 was less than those of the diet calculated based on AIA, which is in agreement with the results of the OB in this study.

Greater values of ATTD were determined by the TC method than by the DIM method (McCarthy et al., 1974; Adeola et al., 1986; Jagger et al., 1992) and the smaller recovery rate of DIM was the main cause of this result (Mroz et al., 1996). Digestibility index marker did not uniformly mix with digesta as DIM passed through the digestive tract, and it might be another reason for the difference between the TC method and DIM method (Adeola, 2001). However, both Moughan et al. (1991) and Kavanagh et al. (2001) reported similar ATTD of GE as determined by the TC method and AIA, where high AIA recovery was obtained (98.2% and 99.9%, respectively).

Digestibility index marker and diet affected DIM recovery in feces. Different fecal recovery of Cr_2O_3, TiO_2 or AIA have been reported (Jagger et al., 1992; Kavanagh et al., 2001). In this study, the relatively short fecal collection period might account for the low DIM recovery and further study is required. The retention of a small amount of Cr_2O_3 in the large intestine may be another reason for the low DIM recovery (Hill et al., 1996).

The recovery of DIM in ileal digesta by the post-valve ‘‘T’’ caecal cannulation method was influenced by fiber type (Köhler et al., 1990) or fiber level (Yin et al., 2000). Similarly, in this study the OB had smaller TiO_2 recoveries in feces than the CB or the CS, which might be explained by the different character of dietary fibers. The change of physiology and increased intestinal fermentation caused by the increased viscosity (Choct et al., 1996) and longer retention time in the large intestine (Kidder and Manners, 1978) of OB might increase the interaction between TiO_2 and OB, as a result, decreasing the DIM recovery.

2.6 Conclusion

The AID of GE or N within each diet was influenced by the choice of DIM; however, the ATTD of GE or N within each diet was less influenced by the choice of DIM; and the digestibility of OB was differently influenced by DIM compared with CS or CB. The OB had a smaller recovery of TiO_2 but not Cr_2O_3 and AIA compared with the CS or CB. This study suggested the DIM should be carefully chosen to determine AID, especially for the diet which is rich in soluble fiber.

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Table 2-1. Ingredient and nutrient composition of diets

	Diet		
Ingredient, g/kg	Corn starch	Corn bran	Oat bran
Ground corn	623.5	623.5	623.5
Soybean meal	150.0	150.0	150.0
Corn starch	100.0	0.0	0.0
Corn bran	0.0	100.0	0.0
Oat bran	0.0	0.0	100.0
Soybean oil	15.0	15.0	15.0
Chromic oxide premix[1]	25.0	25.0	25.0
Titanium dioxide premix[1]	25.0	25.0	25.0
Fossil shell flour premix[1]	25.0	25.0	25.0
Limestone	15.0	15.0	15.0
Monocalcium phosphate	15.0	15.0	15.0
Salt	3.0	3.0	3.0
Vitamin premix[2]	1.5	1.5	1.5
Mineral premix[3]	1.0	1.0	1.0
Selenium premix[4]	0.5	0.5	0.5
Magnesium oxide	0.5	0.5	0.5
Total	1000.0	1000.0	1000.0
Analyzed nutrient and energy (as fed basis)			
Nitrogen, g/kg	18.5	20.1	21.6
Gross energy, MJ/kg	15.9	15.9	16.3
Acid detergent fiber, g/kg[5]	15.8	34.3	32.0
Neutral detergent fiber, g/kg[5]	58.4	138.9	86.3
Ca, g/kg	12.8	12.5	13.3
P, g/kg	6.4	6.4	6.9

[1]A 5 g chromic oxide was added to 20 g ground corn, a 5 g titanium dioxide was added to 20 g ground corn, or a 20 g diatomaceous earth (Perma-Guard, Inc., Bountiful, UT) was added to 5 g ground corn.

[2]Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D_3, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; D-pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B_{12}, 0.02 mg.

[3]Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

[4]Supplied 300 μg of Se per kilogram of diet.

[5]Acid detergent fiber was expressed inclusive of residual ash; neutral detergent fiber was assayed with a heat stable amylase and expressed inclusive of residual ash.

Table 2-2. Apparent ileal digestibility (AID) of energy and nitrogen[1]

			AID	
Diet	DIM	n	Energy	Nitrogen
CS	Cr_2O_3	12	0.775[b]	0.720[b]
CS	TiO_2	12	0.784[a]	0.731[a]
CS	AIA	12	0.761[c]	0.703[c]
CB	Cr_2O_3	12	0.686[m]	0.710[m]
CB	TiO_2	12	0.696[l]	0.720[l]
CB	AIA	12	0.685[m]	0.709[m]
OB	Cr_2O_3	12	0.749[p]	0.717[p]
OB	TiO_2	12	0.750[p]	0.718[p]
OB	AIA	12	0.755[o]	0.724[o]
CS		36	0.773[u]	0.718
CB		36	0.689[w]	0.713
OB		36	0.751[v]	0.720
	Cr_2O_3	36	0.737[y]	0.716[y]
	TiO_2	36	0.743[x]	0.723[x]
	AIA	36	0.734[z]	0.712[z]
SED[2]				
Diet			0.0063	0.0083
DIM			0.0009	0.0009
Diet × DIM[3]			0.0015	0.0016
DIM × Diet[4]			0.0064	0.0084
P-value				
Diet			< 0.001	0.699
DIM			< 0.001	< 0.001
Diet × DIM			< 0.001	< 0.001

[a,b,c]Means for AID of energy or nitrogen in CS with a common superscript are not different at $P < 0.05$.

[l,m]Means for AID of energy or nitrogen in CB with a common superscript are not different at $P < 0.05$.

[o,p]Means for AID of energy or nitrogen in OB with a common superscript are not different at $P < 0.05$.

[u,v,w]Main effect means for AID of energy in CS, CB and OB with a common superscript are not different at $P < 0.05$.

[x,y,z]Main effect means for AID of energy or nitrogen determined with Cr_2O_3, TiO_2, AIA, or TC with a common superscript are not different at $P < 0.05$.

[1]DIM = digestibility index marker; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; CS = corn starch diet; CB = corn bran diet; OB = oat bran diet; AIA = acid-insoluble ash; n = number of observations.

[2]The standard error of difference (SED) of two means for Diet, DIM, Diet × DIM, and DIM × Diet.

[3]The SED for Diet × DIM listed here is only appropriate for comparing DIM means within Diet.

[4]The SED for DIM × Diet listed here is only appropriate for comparing Diet means within DIM.

Table 2-3. Apparent total tract digestibility (ATTD) of energy and nitrogen[1]

			ATTD	
Diet	Method	n	Energy	Nitrogen
CS	Cr_2O_3	12	0.849[b]	0.773[b]
CS	TiO_2	12	0.850[b]	0.773[b]
CS	AIA	12	0.842[b]	0.761[b]
CS	TC	12	0.868[a]	0.802[a]
CB	Cr_2O_3	12	0.779[n]	0.774 [m]
CB	TiO_2	12	0.791[m]	0.786[m]
CB	AIA	12	0.785[mn]	0.781[m]
CB	TC	12	0.815[l]	0.812[l]
OB	Cr_2O_3	12	0.840[pq]	0.788[pq]
OB	TiO_2	12	0.835[q]	0.780[q]
OB	AIA	12	0.848[p]	0.798[p]
OB	TC	12	0.871[o]	0.829[o]
CS		48	0.852[u]	0.777
CB		48	0.793[v]	0.788
OB		48	0.849[u]	0.799
	Cr_2O_3	36	0.823[y]	0.778[y]
	TiO_2	36	0.825[y]	0.780[y]
	AIA	36	0.825[y]	0.780[y]
	TC	36	0.852[x]	0.814[x]
SED[2]				
Diet			0.0054	0.0090
Method			0.0017	0.0024
Diet × Method[3]			0.0030	0.0042
Method × Diet[4]			0.0060	0.0097
P-value				
Diet			< 0.001	0.071
Method			< 0.001	< 0.001
Diet × Method			< 0.001	< 0.001

[a,b]Means for ATTD of energy or nitrogen in CS with a common superscript are not different at $P < 0.05$.

[l,m,n]Means for ATTD of energy or nitrogen in CB with a common superscript are not different at $P < 0.05$.

[o,p,q]Means for ATTD of energy or nitrogen in OB with a common superscript are not different at $P < 0.05$.

[u,v]Main effect means for ATTD of energy in CS, CB and OB with a common superscript are not different at $P < 0.05$.

[x,y]Main effect means for ATTD of energy or nitrogen determined with Cr_2O_3, TiO_2, AIA, or TC with a common superscript are not different at $P < 0.05$.

[1]CS = corn starch diet; CB = corn bran diet; OB = oat bran diet; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; AIA = acid-insoluble ash; TC = total collection method.

[2]The standard error of difference (SED) of two means for Diet, Method, Diet × Method, and Method × Diet.
[3]The SED for Diet × Method listed here is only appropriate for comparing Method means within Diet.
[4]The SED for Method × Diet listed here is only appropriate for comparing Diet means within Method.

Table 2-4. Digestibility index marker recovery (%) in feces[1]

DIM	Diet	n	Recovery
Cr_2O_3	CS	12	87.9
Cr_2O_3	CB	12	83.4
Cr_2O_3	OB	12	80.9
TiO_2	CS	12	88.2[a]
TiO_2	CB	12	88.1[a]
TiO_2	OB	12	78.3[b]
AIA	CS	12	83.4
AIA	CB	12	85.8
AIA	OB	12	85.2
Cr_2O_3		36	84.1
TiO_2		36	84.8
AIA		36	84.8
	CS	36	86.5
	CB	36	85.7
	OB	36	81.5
SED[2]			
Diet			2.53
DIM			0.68
Diet × DIM[3]			1.18
DIM × Diet[4]			2.71
P-value			
Diet			0.119
DIM			0.463
DIM × Diet			< 0.001

[a,b]Means for CS, CB, and OB within TiO_2 with a common superscript are not different at $P < 0.05$.

[1]DIM = digestibility index marker; CS = corn starch diet; CB = corn bran diet; OB = oat bran diet; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; AIA = acid-insoluble ash; digestibility index marker recovery is the ratio of digestibility index marker amount in feces and feed during the total collection of feces period.

[2]The standard error of difference (SED) of two means for Diet, DIM, DIM × Diet, and Diet × DIM.

[3]The SED for Diet × DIM listed here is only appropriate for comparing DIM means within Diet.

[4]The SED for DIM × Diet listed here is only appropriate for comparing Diet means within DIM.

CHAPTER 3. THE COMBINATION OF DIETARY FIBER AND TIME PERIOD AFFECT ILEAL DIGESTIBILITY INDEX MARKER CONCENTRATION IN GROWING PIGS

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3.1 Abstract

This study was conducted to investigate if the concentration patterns of digestibility index marker (DIM) were influenced by dietary fiber (Diet), ileal digesta collection day (Day), and time period (TP). Eighteen barrows fitted with a T-cannula at the end of the ileum were used in a 2-period randomized complete block design. Three corn-soybean meal-based diets were formulated with corn starch (CS), corn bran (CB) or oat bran (OB) at 100 g/kg. All 3 diets contained 3 DIM, which were chromic oxide, titanium dioxide, and acid-insoluble ash (AIA). The ileal digesta was collected every 3 h between 09:00 to 21:00 h with 4 TP on each of the 3 day. Due to the interaction between Diet and TP, diet comparison within TP using appropriate standard error of the difference of two means were discussed. The Ti or AIA concentration on Day 1 was lower than that on Day 3, while there was no difference between Day 2 and Day 3. The Cr concentration was not affected by the Day. The distribution of Cr concentration in ileal digesta of pigs fed CS, CB and OB was similar to that of Ti and AIA irrespective of TP. When comparing OB and CB, OB had greater Ti and AIA concentrations than CB at TP 2, 3 and 4, but OB had similar DIM concentration with CB at TP 1. In conclusion, the Day had limited effect on DIM concentration; the three DIM moved synchronously in diets irrespective of TP; and the Ti and AIA concentrations varied over the OB and CB at TP 2, 3 and 4, but not at TP 1.

Key words: digestibility index marker, corn bran, oat bran, time period

3.2 Introduction

Chromic oxide (Cr_2O_3), titanium dioxide (TiO_2), and acid-insoluble ash (AIA) are the most common digestibility index markers (DIM) used in pigs to determine ileal and total tract digestibility (McCarthy et al., 1974; Jagger et al., 1992). An important assumption is that DIM uniformly mix within the feed and are uniformly excreted in feces to ensure that the collected sample is representative (Adeola, 2001). Hill et al. (1996) indicated that dry matter (DM) digestibility was affected by the sample collection day. The Cr concentration in ileal digesta varied in relation to time of feeding (Jørgensen et al., 1997) and time of collection (Kim and Stein, 2010), and the Cr_2O_3 appearance in the ileum was also affected by fiber in diet (Owusu-Asiedu et al., 2006). However, the comparison of DIM concentration patterns in diets containing different fiber sources across time period (TP) and at different ileal digesta collection day (Day) has not been investigated. The hypothesis of this study is that there is no effect of dietary fiber (Diet), Day, and TP on DIM concentration in ileal digesta. To test this hypothesis, we investigated Cr, Ti, and AIA concentrations in ileal digesta of pigs fed corn starch (CS), corn bran (CB), and oat bran (OB) at 4 TP on each of the 3 Day.

3.3 Materials and Methods

All animal procedures used in this study were approved by the Purdue Animal Care and Use Committee. The formulation of diets, animal housing conditions, sample collection procedures, analytical methods of DM, Cr, Ti, AIA, acid detergent fiber, and neutral detergent fiber were the same as described in Wang et al. (2017).

Three corn-soybean meal-based diets with 623.5 g/kg corn and 150 g/kg soybean meal, which contained 100 g/kg CS, CB or OB, were used and all three diets contained 5 g/kg Cr_2O_3, 5 g/kg TiO_2, and 20 g/kg diatomaceous earth (AIA, Perma-Guard, Inc., Bountiful, UT). Eighteen

cannulated Hampshire × Duroc × Yorkshire × Landrace barrows were surgically fitted with a simple T-cannula at the distal ileum and all pigs were housed individually in stainless steel metabolism crates during the entire experimental period. In the first period, 18 pigs (initial BW 24.2 ± 0.3 kg) were grouped into 6 blocks according to BW and 3 diets were randomly assigned to pigs within each block. In the second period, the same 18 pigs (initial BW 26.9 ± 0.5 kg) were grouped into 6 new blocks according to BW and went through the same procedure except the same pig consumed diets different from the previous period, which provided 12 observations per diet in total.

Daily feed allowance was set as 4% of BW and pigs were fed at 08:00 and 16:00 h in 2 equal meals. There were 7 d of adaptation period, followed by a 3-d total fecal collection period and a 3-d ileal digesta collection period in each experimental period. The ileal digesta were collected every 3 h between 09:00 to 21:00 h with 4 TP where TP 1 = 09:00 to 12:00 h, TP 2 = 12:00 to 15:00 h, TP 3 = 15:00 to 18:00 h, and TP 4 = 18:00 to 21:00 h on Day 1, 2, and 3. Ileal digesta were forced-air dried at 55°C to constant weight and ground to pass through a 0.5-mm screen before analyses.

3.3.1 Chemical Analysis and Calculation

See the chemical analysis in Chapter 2.

3.3.2 Statistical Analysis

Digestibility index marker concentration data was analyzed as a split-split-plot arrangement in a 2-period randomized complete block design. The concentration of Cr, Ti and AIA of the ileal digesta were multivariate responses and they were individually analyzed with the MIXED procedure (SAS Inst. Inc., Cary, NC). Diet, Day and TP were the main-plot, split-plot,

and split-split plot factors, respectively. There was interaction of Diet and TP, therefore the model used was:

$$Y_{ijklmno} = \mu + Diet_i + Day_j + TP_k + (Diet \times TP)_{ik} + Block_l + Animal_m + (Animal \times Day)_{mj} + (Animal \times Period)_{mn} + (Animal \times Period \times Day)_{mnj} + \varepsilon_{ijklmno}$$

where: $Y_{ijklmno}$ = observation, μ = population mean, $Diet_i$ = diet effect (i = 1 to 3), Day_j = ileal digesta collection day effect (j = 1 to 3), TP_k = time period effect (k = 1 to 4), $(Diet \times TP)_{ik}$ = interaction of Diet and TP, $Block_l$ = block effect (l = 1 to 12), $Animal_m$ = animal effect (m = 1 to 18), $(Animal \times Day)_{mj}$ = interaction of Animal and Day, $(Animal \times Period)_{mn}$ = interaction of Animal and Period (n = 1 to 2), $(Animal \times Period \times Day)_{mnj}$ = interaction of Animal, Period and Day, and $\varepsilon_{ijklmno}$ = residual error. In the model, Diet, Day, TP, and (Diet × TP) were fixed effects; Animal, (Animal × Day), (Animal × Period), and (Animal × Period × Day) were random effects. Least squares means were calculated and separated by the SLICE option with Tukey's adjustment. Statistically significant difference was set at $P < 0.05$ and trend was set at $0.05 < P < 0.10$.

3.4 Results and Discussion

There was interaction between Diet and TP for Cr concentration ($P < 0.001$), Ti concentration ($P < 0.001$), and AIA concentration ($P < 0.001$), but there was no other interaction among Diet, Day, and TP ($P > 0.25$). Therefore, the simple effects of the interaction between Diet and TP; and the main effects of Diet, Day, and TP are shown in Table 3-1. Furthermore, standard error of difference (SED) of two means for TP × Diet appropriate for comparing diet means within TP, and SED for Diet × TP appropriate for comparing TP means within diets are listed in Table 3-1. Diet comparison within TP using appropriate SED of two means are discussed below.

For all the 3 DIM, there was no difference of DIM concentration between Day 2 and Day 3. The Ti or AIA concentration on Day 1 was different from that on Day 3, but there was no

difference for Cr concentration between Day 1 and Day 3. Hill et al. (1996) reported that the apparent ileal digestibility of DM determined by total ileal collection in dogs on first day was less than subsequent sample collection days, and the possible reason was the feedback effect from the colon of dogs, which was supported by the fact that the colon fecal material was found only on the first day. However Jagger et al. (1992) found there was no statistical difference in apparent ileal digestibility of amino acids between days of collection. The possible reason for differences based on collection day in this study was the samples collected at TP 1 on Day 1 had higher variance, and as a result the results of Day 1 was affected when all the data on Day 1 was pooled together.

In this study, Cr_2O_3, TiO_2 and AIA were excreted in a similar pattern among the 3 diets within each TP, which indicated that the similar pattern of movement of the 3 DIM through the intestine across the 3 diets. An important assumption about using DIM to determine digestibility is that the DIM should be representative in diet and collected samples. Choct et al. (1996) indicated that the nutrient and the DIM was relatively uniform in the ileal digesta of chickens fed the well-digested diet, but not in diets containing high levels of soluble non-starch polysaccharide. In this study, the ratio of Cr concentration among three diets within each TP was similar to those of Ti, and AIA, which supported that at least the three DIM were relatively uniform in the ileal digesta.

The CS had greater DIM concentration than CB within each TP, but there was no difference in DIM concentration between CS and OB at TP 2, 3, and 4. The possible reason was that compared with CB, the CS and OB has similar fiber components, which is an important character of diets that affect passage rate of digesta (Milton and Demment, 1988; Owusu-Asiedu et al., 2006).

The level of each DIM was the same across the 3 diets, but in the digesta, the OB had greater Ti and AIA concentrations than CB at TP 2, 3, and 4, and greater Cr concentration at TP 2 and 4, whereas similar DIM concentration at TP 1. The major components of OB is water-soluble

fiber (Jacobs et al., 1983), but the CB is rich in cellulose and hemicellulose which are nearly completely insoluble in water (Rose et al., 2010). Because of the different character of fiber, the CB has greater passage rate than OB, and as a result, the CB was digested less than OB in the small intestine during TP 2, 3, and 4. However, Wilfart et al. (2007) indicated that Cr_2O_3 initial appearance in the ileum was around 2 h, which means the TP 1 in the current study represented one meal following an overnight fast. Furthermore, the highest rate of energy absorption happened two hours after ingestion of feed in pigs fed diets with either 100 g or 400 g fiber (Strathe et al., 2008). Therefore, the greater digestion ability shortly after feeding time than during rest time might be the reason that no difference was found between OB and CB during TP 1.

The concentration of AIA in digesta samples was the greatest compared with the Cr and Ti across all diets. The higher concentrations of AIA in the digesta is reasonable because the dietary level of AIA was 2 g/kg, which was 4 times as much as the concentration of each of the other 2 DIM. Furthermore, the Cr_2O_3 and TiO_2 was also determined as part of the AIA (Kavanagh et al., 2001).

3.5 Conclusion

The day of collection had limited effect on DIM concentration in ileal digesta; Cr_2O_3, TiO_2, and AIA moved synchronously in diets irrespective of time period; and the difference of the DIM concentrations based on OB and CB was affected by time period. This study indicated that any of the three DIM was an equivalent choice in digestibility study.

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Table 3-1. Ileal digesta Cr (g/kg), Ti (g/kg), and acid-insoluble ash concentration (AIA, g/kg) from corn starch (CS), corn bran (CB) and oat bran diets (OB) over 4 time periods (TP) during 3 ileal digesta collection day (Day)[1]

Day	TP	Diet	n	Cr	n	Ti	n	AIA
	TP 1	CS	34	13.3[a]	33	12.8[a]	33	104.1[a]
	TP 1	CB	36	10.0[b]	36	9.3[b]	36	73.7[b]
	TP 1	OB	36	11.5[ab]	36	10.6[b]	36	86.4[b]
	TP 2	CS	35	21.0[c]	35	19.1[c]	35	151.0[c]
	TP 2	CB	36	15.2[d]	36	14.0[d]	36	110.5[d]
	TP 2	OB	35	19.4[c]	35	17.7[c]	35	140.5[c]
	TP 3	CS	36	14.5[e]	36	12.6[e]	36	101.0[e]
	TP 3	CB	36	12.2[f]	36	10.5[f]	36	83.7[f]
	TP 3	OB	35	14.4[ef]	35	12.6[e]	35	101.3[e]
	TP 4	CS	35	17.5[g]	35	15.5[g]	35	128.5[g]
	TP 4	CB	36	12.1[h]	36	10.6[h]	36	86.9[h]
	TP 4	OB	36	16.4[g]	35	14.6[g]	35	120.3[g]
1			141	14.5	140	12.7[m]	140	103.8[m]
2			143	14.9	143	13.5[l]	143	108.4[lm]
3			142	15.0	141	13.7[l]	141	109.8[l]
	TP 1		106	11.6[u]	105	10.9[u]	105	88.1[u]
	TP 2		106	18.5[o]	106	16.9[o]	106	134.0[o]
	TP 3		107	13.7[q]	107	11.9[q]	107	95.3[q]
	TP 4		107	15.3[p]	106	13.6[p]	106	111.9[p]
		CS	140	16.6[x]	139	15.0[x]	139	121.2[x]
		CB	144	12.4[z]	144	11.1[z]	144	88.7[z]
		OB	142	15.4[y]	141	13.9[y]	141	112.1[y]
SED[2]								
Day				0.32		0.29		2.35
TP				0.37		0.33		2.71
Diet				0.41		0.34		2.67
TP × Diet[3]				0.70		0.61		4.91
Diet × TP[4]				0.65		0.58		4.78
P-value[5]								
Day				0.194		0.003		0.041
TP				< 0.001		< 0.001		< 0.001
Diet				< 0.001		< 0.001		< 0.001
TP × Diet				< 0.001		< 0.001		< 0.001

[a,b] Means for CS, CB, and OB at TP 1 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[c,d] Means for CS, CB, and OB at TP 2 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[e,f] Means for CS, CB, and OB at TP 3 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[g,h] Means for CS, CB, and OB at TP 4 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[l,m] Main effect means for Day 1, 2, and 3 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[o,p,q,u] Main effect means for TP 1, 2, 3, and 4 with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[x,y,z] Main effect means for CS, CB, and OB with a common superscript are not different at $P < 0.05$ within each digestibility index marker.

[1]TP 1 = 09:00 to 12:00 h; TP 2 = 12:00 to 15:00 h; TP 3 = 15:00 to 18:00 h; TP 4 = 18:00 to 21:00 h; n = number of observations.

[2] The standard error of difference (SED) of two means for Day, TP, Diet, TP × Diet, and Diet × TP.

[3]The SED for TP × Diet listed here is only appropriate for comparing diet means within TP.

[4]The SED for Diet × TP listed here is only appropriate for comparing TP means within diets.

[5] The *P* value for Diet × Day, Day × TP, and Diet × Day × TP were greater than 0.25 and are not shown.

CHAPTER 4. DIGESTIBILITY INDEX MARKER TYPE, BUT NOT INCLUSION LEVEL AFFECTS APPARENT DIGESTIBILITY OF ENERGY AND NITROGEN AND MARKER RECOVERY IN GROWING PIGS REGARDLESS OF ADDED OAT BRAN

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4.1 Abstract

This study was conducted to investigate if the apparent ileal digestibility (AID) of gross energy (GE) or nitrogen (N) was influenced by inclusion level and type of digestibility index marker (DIM) and inclusion level of oat bran (OB), and if the apparent total tract digestibility (ATTD) of GE or DIM recovery was influenced by the three aforementioned factors and duration of feces collection. Six diets were formulated as a 2 × 3 factorial arrangement with two levels of OB (0 or 100 g/kg) and three levels of DIM (2.5, 5.0, or 7.5 g/kg). Both chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) were added to the same diet and their inclusion levels were consistent in each experimental diet. In Exp. 1, eighteen barrows fitted with T-cannulas at the distal ileum were used in a triplicate 6 × 2 incomplete Latin Square design with 6 dietary treatments and 2 periods. The ileal digesta were collected for 3 d after 5-d adaptation, and the AID of GE and N were determined by measuring Cr_2O_3 or TiO_2 in diets and ileal digesta samples. In Exp. 2, a total of 72 barrows were used in a randomized complete block design, and the feces were collected for either 3 or 5 d after a 7-d adaptation according to the assignment. Experimental diets were the same as Exp. 1. The ATTD of GE was determined by both total collection and index methods, and DIM recovery was determined by measuring Cr_2O_3 or TiO_2. In Exp. 1, there was no interaction

among OB level, DIM level, and DIM type for AID of GE and N. The AID of GE and N determined by TiO_2 were greater ($P < 0.05$) than Cr_2O_3 regardless of the OB level and DIM level. Neither the OB level nor the DIM level affected the AID of GE or N. In Exp. 2, no interaction among OB level, DIM level, duration of feces collection, and DIM type for ATTD of GE and DIM recovery was observed. The DIM level and duration of feces collection had no effect on ATTD of GE and DIM recovery. The total collection method resulted in greater ($P < 0.05$) ATTD of GE than TiO_2, which was greater ($P < 0.05$) than that determined by Cr_2O_3. Similarly, the recovery of TiO_2 was greater ($P = 0.007$) than Cr_2O_3. Inclusion of 100 g/kg OB decreased ($P = 0.003$) ATTD of GE but did not affect the recovery of DIM. In conclusion, the AID of GE and N, the ATTD of GE, and the recovery of Cr_2O_3 or TiO_2 were affected by DIM type, but not DIM level; the inclusion of OB had no effect on AID of GE and N, and DIM recovery; and the duration of feces collection had no effect on ATTD of GE, and DIM recovery.

Key words: chromic oxide, digestibility, digestibility index marker, oat bran, swine, titanium dioxide

4.2 Introduction

Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) are two of the important indicators to evaluate the nutritional value or energy in feed ingredients and diets. The addition of digestibility index marker (DIM) as an index to determine digestibility avoids the need for quantitative records of feed intake and feces output (Adeola, 2001), and therefore this index method has been widely used in digestibility studies in non-ruminant animals. Theoretically, the nutrient digestibility values determined by different levels and types of DIM should be accordant. However, various DIM levels, which mostly ranges from 1 to 5 g/kg, and different DIM

types, which mainly includes chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2), have been used and inconsistent results about the effect of DIM level or type have been reported (Yin et al., 2000; Kavanagh et al., 2001; Olukosi et al., 2012). To the best of our knowledge, the verification of this theory using three equally spaced levels of DIM has not been investigated in pigs.

The previous study in our group indicated that the addition of 100 g/kg oat bran (OB) in the diet decreased TiO_2 recovery to 78.5% compared with 88.2% in the diet without OB, which may be due to the relatively short duration of feces collection (Wang et al., 2017). Therefore, the objective of this study was to investigate the effect of DIM level and type on AID of gross energy (GE) and nitrogen (N), and the effect of DIM level and type, and duration of feces collection on ATTD of GE and DIM recovery of diets with or without OB in diets fed to growing pigs.

4.3 Materials and Methods

All animal procedures used in this study were approved by the Purdue University Animal Care and Use Committee.

To be consistent with a previous study (Wang et al., 2017), the OB used in this study was purchased from the same local supplier. Two levels of OB (0 or 100 g/kg) and 3 levels of DIM (2.5, 5.0, or 7.5 g/kg) were used to formulate 6 corn-soybean meal-based diets in a 2 × 3 factorial arrangement (Table 4-1). All diets were formulated to meet or exceed the vitamin and mineral requirement estimates for pigs (NRC, 2012). Both Cr_2O_3 and TiO_2 were added to the same diet as DIM and their inclusion levels were consistent in each experimental diet.

Exp. 1: Apparent Ileal Digestibility of GE and N

Eighteen barrows were surgically fitted with T-cannulas at the distal ileum following the description of Dilger et al. (2004). All pigs were individually housed in stainless steel crates (1.22

× 1.22 m) equipped with a feeder and a nipple drinker. In the first period, 18 pigs (initial BW = 25.8 ± 0.4 kg) were grouped into 3 blocks based on BW, and 6 diets were randomly assigned to pigs within each block. In the second period, the same 18 pigs (initial BW = 27.4 ± 0.5 kg) went through the same procedure except that pigs consumed diets which were different from the first period. Daily feed allowance was set as 4% of the initial smallest BW in each period, and pigs were fed at 0800 and 1700 h in 2 equally divided meals. Water was available at all times during the experiment. Each experimental period consisted of 5 d of adaptation followed by 3 d of ileal digesta collection. Plastic bags containing 10 mL of 10% formic acid was attached to the T-cannula with a rubber O-ring to collect ileal digesta from 0800 to 1700 h on d 6, 7, and 8. The attached bags were inspected every 30 min and the filled bags were changed and stored at –20°C until further processing.

Exp. 2: Apparent Total Tract Digestibility of GE and Digestibility Index Marker Recovery

A total of 72 barrows (initial BW = 21.1 ± 0.2 kg) were used in 3 periods and individually housed in the same crates and environmental conditions with Exp. 1. Pigs were fed one of the 6 experimental diets with a 7-d adaptation period followed by a 3-d or 5-d total collection of feces, which formulated 12 treatments with a 2 × 3 × 2 factorial arrangement. Pigs were allotted to 6 blocks based on BW within periods and assigned to a randomized complete block design with 12 treatments and 6 replicate pigs per treatment. Daily feed allowance was set as 4% of the initial mean BW, and pigs were fed at 0700 and 1700 h in 2 equally divided meals. The feces samples were collected according to the marker-to-marker procedure described by Akinmusire and Adeola (2009) except indigo carmine instead of ferric oxide was used to mark the initiation and end of feces collection. The fecal collection period lasted 3 or 5 d according to treatment, and was initiated

and ended with the appearance of indigo carmine-marked feces. Feces were collected twice daily and the collected feces were stored at –20°C until further processing.

4.3.1 Chemical Analysis and Calculation

The ileal digesta in Exp. 1 and fecal samples in Exp. 2 from each pig during each period were pooled, subsampled, forced-air dried at 55°C to constant weight. Diet, ileal digesta, and fecal samples were ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany). The dry matter (DM) contents of diets, ileal digesta, and fecal samples were determined by drying in a forced-air oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Chromium content was analyzed as described by Saha and Gilbreath (1991) with modifications of heating temperature at 400°C during perchloric acid digestion and standing overnight after dilution with distillated water to 100 mL, and determined by spectrophotometer at 450 nm wavelength (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerlan). Titanium content was analyzed with the method described by Myers et al. (2004) with a modification of heating time. In short, as-fed basis samples were digested in Kjeldahl digestion tubes with the presence of 13 mL concentrated sulfuric acid and catalyst at 420°C for 4 h. Then 10 mL of 30% hydrogen peroxide was added to the cooled digestion solution and the total liquid weight was brought up to 100 g, which stood overnight. The Ti content was determined by the spectrophotometer at 410 nm wavelength. Nitrogen content was determined with the combustion method (TruMac® N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000).

Gross energy content was analyzed by isoperibol bomb calorimeter (Model 6200, Parr Instrument Co., Moline, IL).

The AID and ATTD of GE (Adedokun and Adeola, 2005) and DIM recovery were calculated using the following equations:

$$\text{AID of GE, \%} = [1 - (DIM_{diet}/DIM_{digesta}) \times (GE_{digesta}/GE_{diet})] \times 100,$$

$$\text{ATTD of GE, \%} = [1 - (DIM_{diet}/DIM_{feces}) \times (GE_{feces}/GE_{diet})] \times 100,$$

$$\text{ATTD of GE, \%} = [1 - (GE_{feces} \times W_{feces}) / (GE_{diet} \times W_{diet})] \times 100,$$

$$\text{Recovery of DIM, \%} = (DIM_{feces} \times W_{feces}) / (DIM_{diet} \times W_{diet}) \times 100,$$

where DIM_{diet}, $DIM_{digesta}$, and DIM_{feces} are the DIM concentration of the diet, ileal digesta, and feces, respectively (mg/kg DM); GE_{diet}, $GE_{digesta}$, and GE_{feces} are the GE concentration of the diet, ileal digesta, and feces, respectively (kcal/kg DM); W_{diet} and W_{feces} are the total weight of diet consumption and output of feces during collection period, respectively (kg DM). The AID of N is calculated with GE replaced by N (mg/kg DM).

4.3.2 Statistical Analysis

In Exp. 1, the data on AID were analyzed as a split-plot arrangement, with the dietary treatments as whole-plot factor, and the DIM type as split-plot factor. There was no interaction among factors. The model included OB level, DIM level, and DIM type as fixed effects, and block, the interaction of pig and period, and the interaction of block and DIM type as random effects.

In Exp. 2, the ATTD of GE data were analyzed as a split-plot arrangement, where 2 × 3 × 2 factorial arrangement with 2 levels of OB inclusion, 3 levels of DIM inclusion, and 2 levels of duration of feces collection were whole-plot factors; and the split-plot factor was the method, which refers to a generic term including total collection, Cr_2O_3, and TiO_2. Reduced model was used by excluding the interactions among factors due to the absence of statistical significance. Finally, the fixed effects in the model were OB level, DIM level, duration of feces collection, and method, and the random effects were block, the interaction of block with either one of OB level, DIM level, duration of feces collection, and method and the 3-way interaction of block, OB level, and DIM level.

In Exp. 2, the data on recovery of Cr_2O_3 and TiO_2 were analyzed as a split-plot arrangement, where the whole-plot factor was the same with the analysis of ATTD of GE, and the split-plot factor was DIM type including Cr_2O_3 and TiO_2. Similarly, the interactions among factors were not statistically significant, and the reduced model was used. Finally, the model included OB level, DIM level, duration of feces collection, and DIM type as fixed effects, and the random effects were the same with the model of ATTD of GE except replacing method with DIM type.

The analyzed and calculated concentration of DIM was analyzed using REG procedure of SAS (SAS Inst. Inc., Cary, NC). The rest of the data were analyzed using the MIXED procedure of SAS. Least squares means were separated by PDIFF option with the Tukey's adjustment. The experimental unit was pig in all the analyses, and the statistical significance was declared at $P < 0.05$.

4.4 Results

During the whole experimental process, one pig in Exp. 2 (with the treatment of OB level as 0, DIM level as 7.5 g/kg, and duration of fecal collection as 5 d) had diarrhea and was removed from experiment.

The analyzed Cr or Ti concentration in experimental diets was in agreement with the calculated value of Cr or Ti (Figure 4-1), respectively. The slope of the regression line of dietary Cr concentration was 0.884, and the R^2 and root of mean squared error of the model were 0.999 and 0.0388, respectively. In terms of dietary Ti concentration, the slope of the regression line was 0.982, and the R^2 and root of mean squared error of the model were 0.999 and 0.0403, respectively.

The result of AID of GE and N in Exp. 1 are presented in Table 4-2. No interaction among OB level, DIM level, and DIM type in the model of AID of GE or N was observed. The AID of

GE and N were unaffected by either OB level or DIM level among the 6 experimental diets. However, the choice of DIM type affected the results of AID of GE (74.1% and 73.1% for TiO_2 and Cr_2O_3, respectively; $P = 0.049$) and N (72.7% and 71.7% for TiO_2 and Cr_2O_3, respectively; $P = 0.042$), where TiO_2 determined greater values than Cr_2O_3.

The ATTD of GE in Exp. 2 is shown in Table 4-3 and there was no interaction among OB level, DIM level, duration of feces collection, and method. The result of DIM recovery is presented in Table 4-4 and no interaction among OB level, DIM level, duration of feces collection, and DIM type was observed. Digestibility index marker level and duration of feces collection did not affect the ATTD of GE and DIM recovery. The TiO_2 determined greater ($P < 0.05$) ATTD of GE than Cr_2O_3, while the greatest ATTD of GE was determined by the total collection method ($P < 0.05$). Including 100 g/kg OB in diet decreased ($P = 0.003$) the ATTD of GE, but did not affect the DIM recovery. Furthermore, the DIM recovery of TiO_2 at 83.4% was greater ($P = 0.007$) than 78.9% for Cr_2O_3.

4.5 Discussion

The effect of inclusion level of DIM in diet on nutrient digestibility has been investigated in swine and poultry (Jagger et al., 1992; Olukosi et al., 2012). Based on the assumption about DIM, that DIM should be non-absorbable, indigestible, and completely voided in the feces (Adeola, 2001), the results determined by adding different inclusion levels of DIM should not be different. However, the validation of this theory might be related to animal species because of differences in the gastrointestinal tract. Olukosi et al. (2012) reported that the AID of His, Trp, Cys, and Pro in corn-soybean meal-based diets were greater at 3 g/kg DIM concentration than 5 g/kg in broilers, either using Cr_2O_3 or TiO_2. In addition, increased digestibility with decreasing levels of Cr_2O_3

from 20 to 5 g/kg in tilapia was observed (Shiau and Liang, 1995). But similar results of AID of N and amino acids between 3 and 5 g/kg DIM concentration were observed by Olukosi et al. (2012) in barrows. This is consistent with the results in the current study, where the AID of GE or N, ATTD of GE, and DIM recovery were not affected by three dietary DIM levels in barrows.

In the current study, the DIM recovery results were consistent with the results of ATTD of GE, which indicated that the lower recovery of Cr_2O_3 might be a reason for lower ATTD of GE determined by Cr_2O_3 than that determined by TiO_2. The equation used to calculate the recovery of DIM in this study is: Recovery of Cr_2O_3, % = $(Cr_{feces} \times W_{feces}) / (Cr_{diet} \times W_{diet}) \times 100$; Recovery of TiO_2, % = $(Ti_{feces} \times W_{feces}) / (Ti_{diet} \times W_{diet}) \times 100$. In the equation for calculating the recovery of Cr_2O_3 and TiO_2, the weight of feces and feed intake were the same for both Cr_2O_3 and TiO_2, but the recovery of TiO_2 was greater than that of Cr_2O_3. This indicated that Ti_{feces}/Ti_{diet} was greater than Cr_{feces}/Cr_{diet}. The equation used to calculate ATTD of GE in this study is: ATTD of GE, % = $[1 - (Cr_{diet}/Cr_{feces}) \times (GE_{feces}/GE_{diet})] \times 100$ for Cr_2O_3 as DIM; ATTD of GE, % = $[1 - (Ti_{diet}/Ti_{feces}) \times (GE_{feces}/GE_{diet})] \times 100$ for TiO_2 as DIM. In the equation for calculating the ATTD using Cr_2O_3 or TiO_2, the GE of feces and feed intake were the same, but the ATTD determined by TiO_2 was greater than that by Cr_2O_3. This indicated that Ti_{diet}/Ti_{feces} was less than Cr_{diet}/Cr_{feces}, which is consistent with the result of marker recovery. The lower recovery of Cr_2O_3 compared with TiO_2 compares well with several previous studies (Jagger et al., 1992; Yin et al., 2000; Olukosi et al., 2012), but is inconsistent with Köhler et al. (1990), where higher recovery of Cr_2O_3 than TiO_2 was observed. The factors that cause the different recovery of TiO_2 from Cr_2O_3 are various, but one of the possible and reasonable reasons is the accuracy of chemical analysis of the marker. Firstly, the method used in quantifiable measurements of both Cr and Ti in the current study is colorimetry, but the stability of the color forming compounds in the chemical analysis of Cr and Ti are different.

The intense orange or yellow color of peroxytitanic acid is formed when TiO_2 reacts with hydrogen peroxide which stays stable for at least 9 wk (Short et al., 1996; Myers et al., 2004). However, chromic acid is formed when Cr_2O_3 reacts with perchloric acid (Fenton and Fenton, 1979), and it readily polymerizes by loss of water to form dichromic acid and higher polymers (Udy, 1956), which indicates the stability of the reaction of Cr is relatively low compared with that of Ti. Furthermore, the loss of Cr during digestion with the formation of volatile chlorine compounds (Gorsuch, 1959) and the fusion or bonding of Cr to glassware (Cary and Allaway, 1971) could also contribute to the low recovery of Cr_2O_3. Moreover, Jagger et al. (1992) indicated that the standard error for AID values associated with TiO_2 was lower than that of Cr_2O_3.

The current study found that more accurate analysis of Ti was achieved than Cr, where the analyzed concentrations of Ti in diets were in better match with the expected values than Cr. Williams et al. (1962) found that silicate, Al, Ca, and Mg in aqueous solution interfered in the determination of Cr with atomic absorption spectroscopy, and later Saha and Gilbreath (1991) indicated that this type of interference conceivably could happen in colorimetric determination, which was used in this study. It is worth noting that TiO_2 was not digested during the chemical digestion process of Cr_2O_3, but the undissolved TiO_2 might interfere with the absorbance of Cr solution with the spectrophotometer. Both Olukosi et al. (2012) and Myers et al. (2004) indicated that the chemical analysis of Ti determined by colorimetry in diet or bovine fecal samples was not affected when Cr was present, but whether the determination of Cr was affected in presence of TiO_2 is still unknown.

The total collection method determined greater ATTD of GE than DIM method, which is in an agreement with previous studies (McCarthy et al., 1974; Adeola et al., 1986; Jagger et al., 1992) and the lower recovery of DIM might be the reason. In the current study, the DIM recovery

ranged from 77.2% to 80.7% for Cr_2O_3 and 80.1% to 87.2% for TiO_2, which were within the range of literature values (Ishikawa and Sugimura, 1973; Köhler et al., 1990; Jagger et al., 1992; McClean, 1993; Wang et al., 2017). The factors that might contribute to the low DIM recovery have been recognized and investigated for decades but there is still not an accordant conclusion. Compared with the content of other components, the relatively small proportion of DIM in the experimental diets, which ranged from 2.5 to 7.5 g/kg in the current study, might partly explain the low DIM recovery (Köhler et al., 1990). On the other hand, perfectly accurate records of intake of the experimental diets is difficult in practice, especially when the diet is fed in mash form. Relatively higher TiO_2 recovery was reported by Peddie et al. (1982), where pelleted diet was fed to mature laying hens. Except for the two externally practical factors, the physical or biochemical interactions between markers and pigs could also explain the low marker recovery. The fineness of DIM and the tortuous intestine structure of non-ruminant animals may cause the retention of DIM in the intestine (McClean, 1993; Hill et al., 1996; Mroz et al., 1996). The possibility of biochemical reactions of Cr or Ti in the animal body, as discussed by Olukosi et al. (2012), indicated the absorption of DIM and participation of reaction.

In the current study, the effect of the duration of feces collection on ATTD of GE and DIM recovery was investigated because the relatively short duration of feces collection might be the reason of the low recovery of DIM in the previous study (Wang et al., 2017). And at least 4 days of total collection of feces was recommended by Adeola (2001). But the results obtained from this study indicated that there was no difference between 3 or 5 d of feces collection and it was concluded that the duration of feces collection may not be one of the reasons for low marker recovery.

4.6 Conclusion

In conclusion, the choice of DIM type, but not marker level affected the AID of GE and N, the ATTD of GE, and the marker recovery. The inclusion of 100 g/kg OB did not affect the AID of GE and N and DIM recovery. The duration of feces collection had no effect on ATTD of GE and DIM recovery.

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Table 4-1. Ingredient composition and analyzed nutrient concentration of experimental diets, g/kg as-fed basis[1]

	Oat bran, g/kg:	0			100		
Item	DIM, g/kg:	2.5	5.0	7.5	2.5	5.0	7.5
Ingredient							
Ground corn		620.0	595.0	570.0	620.0	595.0	570.0
Soybean meal, 48% crude protein		203.5	203.5	203.5	203.5	203.5	203.5
Oat bran		0.0	0.0	0.0	100.0	100.0	100.0
Cornstarch		100.0	100.0	100.0	0.0	0.0	0.0
Titanium dioxide premix[2]		12.5	25.0	37.5	12.5	25.0	37.5
Chromic oxide premix[3]		12.5	25.0	37.5	12.5	25.0	37.5
Soybean oil		15.0	15.0	15.0	15.0	15.0	15.0
Limestone		15.0	15.0	15.0	15.0	15.0	15.0
Monocalcium phosphate		15.0	15.0	15.0	15.0	15.0	15.0
Salt		3.0	3.0	3.0	3.0	3.0	3.0
Vitamin premix[4]		1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[5]		1.0	1.0	1.0	1.0	1.0	1.0
Selenium premix[6]		0.5	0.5	0.5	0.5	0.5	0.5
Magnesium oxide		0.5	0.5	0.5	0.5	0.5	0.5
Total		1,000	1,000	1,000	1,000	1,000	1,000
Analyzed nutrient							
Dry matter		89.1	88.5	88.6	88.5	88.9	88.7
Gross energy (kcal/g)		3.86	3.95	3.83	3.91	3.91	3.83
Nitrogen		2.46	2.47	2.42	2.75	2.66	2.63
Cr		1.64	3.09	4.65	1.66	3.18	4.70
Ti		1.49	2.99	4.41	1.52	3.04	4.48

[1]DIM = digestibility index marker.

[2]A 5 g titanium dioxide was added to 20 g ground corn.

[3]A 5 g chromic oxide was added to 20 g ground corn.

[4]Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D_3, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; D-pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B_{12}, 0.02 mg.

[5]Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

[6]Supplied 0.3 mg Se/kg of diet.

Table 4-2. Influence of oat bran level, digestibility index marker level and type on apparent ileal digestibility (AID) of energy and nitrogen in experimental diets fed to pigs in Exp. 1[1]

					AID, %	
Item	OB level, g/kg	DIM level, g/kg	DIM type	*n*	Energy	Nitrogen
	0	2.5	Cr_2O_3	6	73.2	73.1
	0	2.5	TiO_2	6	73.8	73.6
	0	5.0	Cr_2O_3	6	74.1	72.5
	0	5.0	TiO_2	6	75.4	73.8
	0	7.5	Cr_2O_3	6	72.1	67.7
	0	7.5	TiO_2	6	73.0	68.8
	100	2.5	Cr_2O_3	6	72.8	71.7
	100	2.5	TiO_2	6	73.7	72.6
	100	5.0	Cr_2O_3	6	74.1	73.3
	100	5.0	TiO_2	6	75.4	74.6
	100	7.5	Cr_2O_3	6	72.1	71.9
	100	7.5	TiO_2	6	73.1	72.9
			Cr_2O_3	36	73.1	71.7
			TiO_2	36	74.1	72.7
SED[2]						
OB level					1.11	1.73
DIM level					1.36	2.12
DIM type					0.39	0.40
P-value[3]						
OB level					0.962	0.472
DIM level					0.290	0.299
DIM type					0.049	0.042

[1]OB = oat bran; DIM = digestibility index marker; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; n = number of observations.

[2]SED = standard error of difference.

[3]The *P*-values for interactions including OB level × DIM level, OB level × DIM type, DIM level × DIM type, and OB level × DIM level × DIM type are all > 0.10 and the *P*-values are not presented.

Table 4-3. Influence of oat bran level, digestibility index marker level, method, and duration of feces collection on apparent total tract digestibility (ATTD) of energy in experimental diets fed to pigs in Exp. 2[1]

Item	OB level, g/kg	DIM level, g/kg	Method	*n*[2]	ATTD of energy, %
	0	2.5	Cr_2O_3	12	84.6
	0	2.5	TiO_2	12	86.0
	0	2.5	TC	12	87.9
	0	5.0	Cr_2O_3	12	84.0
	0	5.0	TiO_2	12	84.6
	0	5.0	TC	12	87.7
	0	7.5	Cr_2O_3	11	84.7
	0	7.5	TiO_2	11	85.1
	0	7.5	TC	11	87.7
	100	2.5	Cr_2O_3	12	83.3
	100	2.5	TiO_2	12	84.9
	100	2.5	TC	12	86.9
	100	5.0	Cr_2O_3	12	83.1
	100	5.0	TiO_2	12	84.0
	100	5.0	TC	12	86.9
	100	7.5	Cr_2O_3	12	82.6
	100	7.5	TiO_2	12	83.1
	100	7.5	TC	12	86.0
	0			105	85.8
	100			108	84.5
			Cr_2O_3	71	83.7[z]
			TiO_2	71	84.6[y]
			TC	71	87.2[x]
SED[3]					
OB level					0.24
DIM level					0.39
Duration of feces collection[4]					0.34
Method					0.18
P-value[5]					
OB level					0.003
DIM level					0.231
Duration of feces collection					0.109
Method					< 0.001

[x,y,z]Main effect means for method within a column without a common superscript letter differ ($P < 0.05$).

[1]OB = oat bran; DIM = digestibility index marker; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; TC = total collection.

[2]n = number of observations; it includes 3 and 5-d duration of feces collection.
[3]SED = standard error of difference.
[4]Duration of feces collection refers total collection of feces for either 3 or 5 d after a 7-d adaptation. Method refers the use of total collection, Cr_2O_3, or TiO_2 to determine digestibility.
[5]The *P*-values for all the interactions including OB level, DIM level, duration of feces collection, or method are all > 0.10 and the *P*-values are not presented.

Table 4-4. Influence of oat bran level, digestibility index marker level and type, and duration of feces collection on recovery of digestibility index markers of pigs fed experimental diets in Exp. 2[1]

Item	OB level, g/kg	DIM level, g/kg	DIM type	*n*[2]	Recovery, %
	0	2.5	Cr_2O_3	12	78.9
	0	2.5	TiO_2	12	86.1
	0	5.0	Cr_2O_3	12	77.2
	0	5.0	TiO_2	12	80.1
	0	7.5	Cr_2O_3	11	80.7
	0	7.5	TiO_2	11	82.6
	100	2.5	Cr_2O_3	12	78.9
	100	2.5	TiO_2	12	87.2
	100	5.0	Cr_2O_3	12	77.4
	100	5.0	TiO_2	12	81.9
	100	7.5	Cr_2O_3	12	80.5
	100	7.5	TiO_2	12	82.6
			Cr_2O_3	71	78.9
			TiO_2	71	83.4
SED[3]					
OB level					1.56
DIM level					1.66
Duration of feces collection[4]					1.03
DIM type					1.03
P-value[5]					
OB level					0.782
DIM level					0.129
Duration of feces collection					0.322
DIM type					0.007

[1]OB = oat bran; DIM = digestibility index marker; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; digestibility index marker recovery is the ratio of digestibility index marker amount in feces and feed during the total collection of feces period.

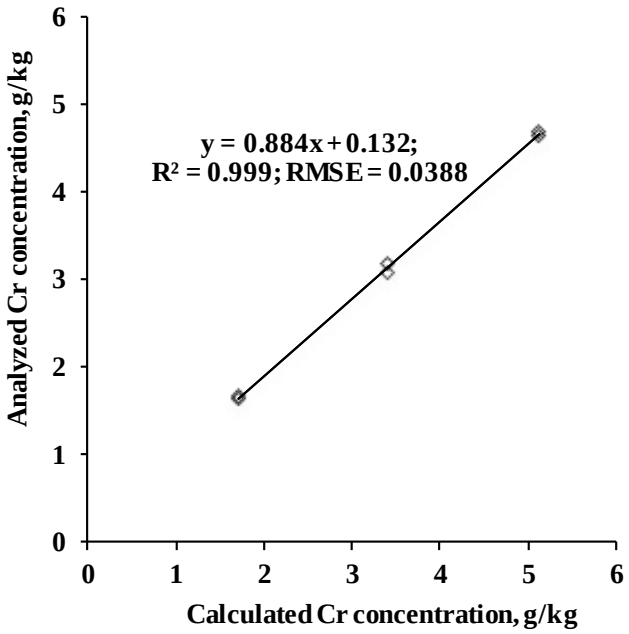
[2]n = number of observations; it includes 3-d and 5-d duration of feces collection.

[3]SED = standard error of difference.

[4]Duration of feces collection refers total collection of feces for either 3 or 5 d after a 7-d adaptation.

[5]The *P*-values for all the interactions including OB level, DIM level, duration of feces collection, or DIM type are all > 0.10 and the *P*-values are not presented.

A.



B.

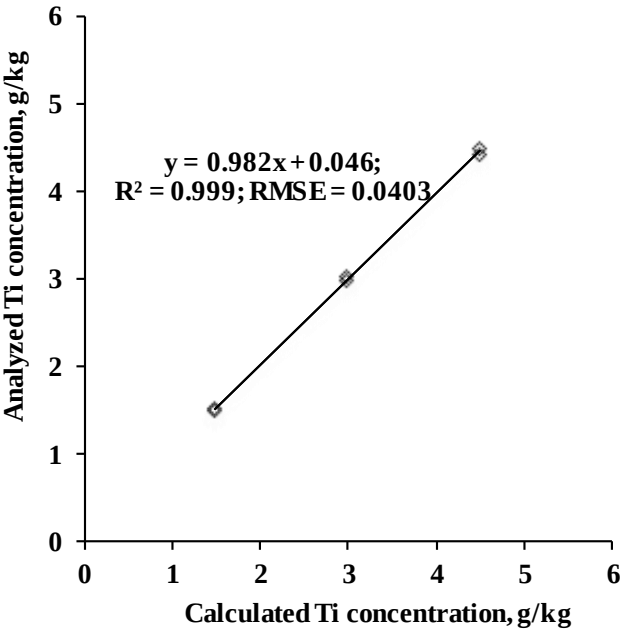


Figure 4-1. The regression analysis between analyzed and calculated Cr (Panel A) and Ti concentrations (Panel B) in experimental diets. Each point represents the mean value of duplicated measurements. The chromic oxide and titanium dioxide were included at 2.5, 5.0, and 7.5 g/kg in experimental diets. RMSE= Root of mean squared error.

CHAPTER 5. ADDITIVITY OF APPARENT AND STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACID DETERMINED BY CHROMIC OXIDE AND TITANIUM DIOXIDE IN MIXED DIETS CONTAINING WHEAT AND MULTIPLE PROTEIN SOURCES FED TO GROWING PIGS

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5.1 Abstract

The aim of this study was to investigate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in mixed diets containing wheat, canola meal (CM), meat and bone meal (MBM), and sorghum distillers' dried grains with solubles (DDGS) fed to pigs with Cr_2O_3 and TiO_2 as digestibility index markers (DIM). Four diets were prepared to contain wheat, CM, MBM, or DDGS as a sole source of N; three mixed diets were prepared to contain wheat, CM, and MBM; wheat, MBM, and DDGS; or wheat, CM, MBM, and DDGS; also, a N-free diet was prepared to estimate the basal ileal endogenous losses (BEL) of CP and AA. Both chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) were incorporated at 5 g/kg into each diet. Sixteen barrows (initial BW = 34.7 ± 0.6 kg) surgically fitted with T-cannulas at the distal ileum were allotted to a duplicate 8 × 4 Youden square design with eight experimental diets and four periods. During each 7-d period, the ileal digesta was collected for 2 d after a 5-d adaptation, and the AID and SID of CP and AA were determined using Cr_2O_3 or TiO_2 as DIM. There was no interaction between diet and DIM type for both AID and SID of CP and AA. Similar BEL, AID, and SID of CP and AA were determined by Cr_2O_3 and TiO_2. In the wheat-CM-MBM diet, the measured AID of CP and most AA determined with Cr_2O_3 or TiO_2

were not different from the predicted values, which was determined based on the measured AID values in wheat, CM, and MBM. In the wheat-MBM-DDGS diet, the measured and predicted AID of CP and seven indispensable AA differed ($P < 0.05$) using Cr_2O_3 as DIM, and the measured and predicted AID of CP and four indispensable AA differed ($P < 0.05$) using TiO_2 as DIM. The measured AID of CP and most indispensable AA were greater ($P < 0.05$) than predicted AID regardless of DIM type for the wheat-CM-MBM-DDGS diet. The measured SID of CP and indispensable AA were consistent with the predicted values, except Lys in the wheat-CM-MBM diet using either DIM and His in the wheat-MBM-DDGS diet with Cr_2O_3 as DIM. In conclusion, more accurate prediction of ileal digestibility of CP and AA was achieved using SID rather than AID in mixed diets containing wheat, CM, MBM, and DDGS. The determination of endogenous loss, AID, and SID of CP and AA were not affected by DIM type in this study. In addition, the additivity of AID and SID of CP and most indispensable AA in mixed diets was not affected by DIM type.

Key words: additivity, chromic oxide, ileal digestibility, swine, titanium dioxide

5.2 Introduction

The application of additive ileal digestibility of amino acids (AA) in feed ingredients is fundamental to match AA supply in diets to AA requirements for pigs (Jansman et al., 2002). The standardized ileal digestible AA is adopted to express both requirements for pigs and ingredient contents used in feed formulation (NRC, 2012). Compared with apparent ileal digestibility (AID) of AA, the standardized ileal digestibility (SID) accounts for basal ileal endogenous losses (BEL) of AA; therefore, SID is independent of dietary AA contents and more additive in mixed diets fed to pigs, especially for diets containing low-protein cereal grains (Stein et al., 2007). Lack of additivity of AID has been reported in mixed diets containing corn in pigs (Stein et al., 2005; Xue

et al., 2014); however, the additivity of AID in mixed diets containing wheat is still not clear. According to NRC (2012), the crude protein (CP) content in soft red wheat is 2.7% greater than yellow dent corn, and this difference in CP content might elicit a relatively large change of AID in ingredients (Fan et al., 1994), as well as the additivity of AID in mixed diets.

One of the general approaches to determine ileal digestibility of CP and AA is to include digestibility index marker (DIM) in diets and collect representative ileal digesta from pigs fitted with simple T-cannulas (Nyachoti et al., 1997a; Gabert et al., 2001). Chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2), as two of the most common DIM have been reported to affect the determination of ileal digestibility (Kavanagh et al., 2001; Wang et al., 2017), and this might relate to the variant recovery of these two DIM in ileal digesta of pigs (Yin et al., 2000). Therefore, the choice of DIM could affect the determination of ileal digestibility, and consequently, the additivity of ileal digestibility of CP and AA in mixed diets. In previous studies that investigated the assumption of additivity of AID and SID of CP and AA in mixed diets, Cr_2O_3 has been generally used as DIM (Fan et al., 1993; Stein et al., 2005; Xue et al., 2014); however, there is a scarcity of data with TiO_2 as DIM. Thus, the objective of this experiment was to test the null hypothesis that 1) additivity of AID of CP and AA in mixed diets containing wheat, CM, MBM, and DDGS is not different from SID; 2) the determination of AID, SID, and BEL of CP and AA as well as additivity of ileal digestibility is not affected by the choice of DIM.

5.3 Materials and Methods

All animal procedures used in this study were approved by the Purdue University Animal Care and Use Committee.

Eight experimental diets including one nitrogen-free diet (NFD) were formulated (Table 5-1). Four semipurified diets consisted one of wheat, canola meal (CM), meat and bone meal

(MBM), or sorghum distillers' dried grains with solubles (DDGS) as the sole source of CP and AA to measure the AID and SID of CP and AA in test ingredients, which were used to determine the predicted AID and SID of CP and AA in mixed diets. Three mixed diets including wheat-CM-MBM, wheat-MBM-DDGS, and wheat-CM-MBM-DDGS diets were formulated to contain approximately 17% CP and measure the AID and SID of CP and AA in mixed diets. Wheat and MBM contents were the same across the three mixed diets, but the CM and DDGS contents varied among the three mixed diets. All diets were formulated to meet or exceed the estimated vitamin and mineral requirements suggested in NRC (2012). Both Cr_2O_3 and TiO_2 were incorporated into experimental diets as the DIM at the level of 5 g/kg, respectively.

Sixteen barrows (initial BW = 34.7 ± 0.6 kg) were surgically fitted with T-cannulas at the distal ileum as described by Dilger et al. (2004). Pigs were allotted to two blocks with BW as blocking factor and assigned to a duplicate 8 × 4 Youden square design with eight dietary treatments and four experimental periods. All pigs were individually housed in stainless steel crates (1.22 × 1.22 m) equipped with a feeder and a nipple drinker. Daily feed allowance was set as 4% of the initial smallest BW in each block in each period, and pigs were fed at 0800 and 1700 h in two equally divided meals. Water was available at all time during the experiment. In each period, there were 5 d of adaptation and 2 d of ileal digesta collection. On day 6 and 7 of each experimental period, Whirl-Pak bags (Nasco, Fort Atkinson, WI) containing 10 mL of 10% formic acid was attached to the T-cannula with a rubber O-ring to collect ileal digesta from 0800 to 1700 h. The attached bags were inspected every 30 min and the filled bags were changed and stored at –20°C until further processing. The ileal digesta from each pig during each period were pooled, subsampled, and lyophilized.

5.3.1 Chemical Analysis and Calculation

Ingredients, experimental diets, and ileal digesta samples were ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany). The concentrations of dry matter (DM) in ingredients, diets, and ileal digesta samples were measured by drying in a forced-air drying oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). For the ingredients, ether extract was analyzed without acid hydrolysis [method 920.39 (A); AOAC, 2006], and acid detergent fiber [method 973.18 (AD); AOAC, 2006] and neutral detergent fiber (Van Soest et al., 1991) contents were analyzed by a fiber analyzer (ANKOM A2000 Fiber Analyzer, ANKOM Technology, Macedon, NY). Nitrogen contents in ingredients, diets, and ileal digesta samples were determined by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP content was calculated by multiplying 6.25 by the content of N. Amino acid analyses [method 982.30 E (a, b, c); AOAC, 2006] for the diets, ingredients, and ileal digesta samples were performed by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). The concentrations of Cr and Ti were analyzed as described by Saha and Gilbreath (1991) and Myers et al. (2004), respectively, with modifications as described by Wang and Adeola (2018).

The measured AID, BEL, and SID of CP and AA were determined using the following equations (Park et al., 2017):

$$AID\ (\%) = [1 - (DIM_i/DIM_o) \times (AA_o/AA_i)] \times 100;$$

$$BEL\ (g/kg\ DMI) = AA_o \times (DIM_i/DIM_o);$$

$$SID\ (\%) = AID + (BEL/AA_i) \times 100;$$

where DIM_i and DIM_o are DIM concentration (g/kg DM) of diet and ileal digesta, respectively; AA_i and AA_o are the CP or AA concentration (g/kg DM) of diet and ileal digesta, respectively.

The average BEL of CP and AA were calculated from the eight pigs that received the NFD to determined SID from AID.

The predicted AID or SID of CP and AA in mixed diets were determined by the measured AID or SID of CP and AA in ingredients using the following equations (Xue et al., 2014):

$$AID_p\ (\%) = [(CP_W \times AID_W) + (CP_{CM} \times AID_{CM}) + (CP_{MBM} \times AID_{MBM}) + (CP_{DDGS} \times AID_{DDGS})]/(CP_W + CP_{CM} + CP_{MBM} + CP_{DDGS}),$$

where AID_p is the predicted AID of CP in a mixed diet; CP_W, CP_{CM}, CP_{MBM}, and CP_{DDGS} are the concentrations (%) of CP in the mixed diets originating from wheat, CM, MBM, and DDGS, respectively, which were determined by multiplying the concentration of CP (%) in respective ingredients by the proportion (%) of the ingredient in the mixed diet; AID_W, AID_{CM}, AID_{MBM}, and AID_{DDGS} are the measured AID (%) of CP in wheat, CM, MBM, and DDGS, respectively. The predicted AID of each AA in the mixed diets was determined using the same equation by replacing CP with AA in the above equation. The predicted SID of CP or each AA in the mixed diets was determined using the same equation with AID by replacing the AID with SID (%) of CP or each AA in wheat, CM, MBM, and DDGS. The differences between measured and predicted values of AID or SID of CP and AA in the mixed diets were determined with both Cr_2O_3 and TiO_2 as DIM.

5.3.2 Statistical Analysis

The data on AID and SID of CP and AA were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a split-plot arrangement, with dietary treatment as whole-plot factor and the DIM type as split-plot factor. The model included diet, DIM type, and the interaction between diet and DIM type as fixed effects; and pig, period, and interactions between pigs and period, pig and DIM, and period and DIM as random effects. The BEL data were analyzed using the MIXED procedure with DIM type as fixed effect; and pig, period, and interaction between pig

and DIM as random effects. One-sample, two-tailed *t*-test was conducted by the TTEST procedure of SAS to test the null hypothesis that the difference between measured and predicted AID or SID of CP and AA in mixed diets is equal to 0. The ileal digestibility was underestimated when the difference between measured and predicted values was significantly greater than 0 or was overestimated when the difference was significantly less than 0. The experimental unit was the pig in all the analyses, and statistical significance was declared at $P < 0.05$.

5.4 Results

All pigs were in good condition throughout the four periods of the experiment. Among the four feed ingredients used in this study, the concentration of CP ranged from 11.19% in wheat to 60.31% in MBM and similarly, the Lys concentration ranged from 0.36% in wheat to 2.73% in MBM (Table 5-2). Except for the NFD and wheat diet, the other experimental diets contained approximately 17% CP (Table 5-3).

There were no differences in the BEL of CP and all AA determined with Cr_2O_3 or TiO_2 in pigs fed the NFD (Table 5-4). There was no interaction between experimental diet and DIM type for both AID and SID of CP and AA, however, the values of AID and SID of CP and AA determined by both DIM are presented in Tables 5-5 and 5-6. The AID of CP, DM, and AA, and SID of CP and AA in each experimental diet were not affected by the choice of DIM.

In the wheat-CM-MBM diet, the measured AID of His, Lys, Trp, and Tyr determined with either DIM and AID of Thr, Asp, and Pro determined with TiO_2 were greater ($P < 0.05$) than predicted values (Table 5-7). In the wheat-MBM-DDGS diet, the measured AID of CP, Arg, His, Lys, Trp, Pro, and Tyr determined with either DIM and those of Ile, Leu, and Met determined with Cr_2O_3 were greater ($P < 0.05$) than predicted values. Regardless of DIM type, the AID of CP and

all indispensable AA except Arg and Val in the wheat-CM-MBM-DDGS diet were underestimated, where the predicted AID was lower ($P < 0.05$) than the measured AID.

The measured SID of Lys determined with either DIM and SID of Gly determined with Cr_2O_3 were different ($P < 0.05$) from predicted SID in the mixed diet containing wheat, CM, and MBM (Table 5-8). For the wheat-MBM-DDGS diet, the measured SID of His determined with Cr_2O_3 and those of Pro and Tyr determined with either DIM were greater ($P < 0.05$) than corresponding predicted SID. The measured and predicted SID of all AA except Cys and Tyr differed ($P < 0.05$) regardless of DIM in the wheat-CM-MBM-DDGS diet.

5.5 Discussion

The nutrient composition in wheat, CM, and MBM in this study were within the range of reported values for these ingredients (Olukosi and Adeola, 2009; Urriola et al., 2009; NRC, 2012; Xue et al., 2014). Except for the wheat diet, the CP contents in semipurified and mixed diets met the requirement for pigs (NRC, 2012).

The BEL of CP and AA determined with either Cr_2O_3 or TiO_2 in NFD in this study were within the range of values reported in previous studies (Zhai and Adeola, 2011; NRC, 2012; Park et al., 2013; Xue et al., 2014). Value for BEL of AA could be determined by several methods; however, feeding NFD is most widely used due to its simplicity (Adeola, 2001). Although the NFD method is straightforward, several studies have investigated the factors related to diet formulation or physiological status of the pig, which might affect the determination of BEL values in pigs (Park et al., 2013; Kong et al., 2014). This study indicated that the determination of BEL of CP and AA were not affected by the choice of DIM used in NFD. It is worth noting that the AID of DM was not affected by DIM type, which therefore excludes the possibility for other errors that may result in a lack of additivity. Similar to the determination of AID of DM, the choice of

DIM had no effect on the determination of AID and SID of CP and AA in all semipurified and mixed diets in this study, even with high dietary fiber. In corn-soybean meal-based and barley-wheat-soybean meal-based complete diets, however, greater AID of CP was determined with TiO_2 compared with Cr_2O_3 (Jagger et al., 1992; Wang and Adeola, 2018). It has been reported that starch-enriched diets empty faster from the stomach compared with wheat bran- and sugar beet pulp-enriched diets (Guerin et al., 2001). Therefore, it is reasonable to speculate that the different effect of DIM on digestibility between current and previous studies might be caused by discrepant basal components in diets, which was corn starch in this study.

One of the advantages of using SID instead of AID of CP and AA in diet formulation for pigs is that SID is relatively additive in mixtures of feed ingredients (Furuya and Kaji, 1991; Nyachoti et al., 1997b; Stein et al., 2005; Xue et al., 2014). This is because SID has been corrected for the contribution of BEL of CP and AA in ileal digesta, and consequently SID is not affected by dietary CP content (Jansman et al., 2002). Similarly, this conclusion is also supported by the data from the three mixed diets in this study, where the differences between measured and predicted ileal digestibility values of CP and indispensable AA differed from zero in 39 for AID and 3 for SID of 66 observations.

The AID of His, Lys, Trp, and Tyr were not additive in all the three mixed diets, and the possible reason for this might be the low concentrations of these AA in the mixed diets, which could result in greater errors in the measurement of these AA. Cereal grains, such as corn and wheat, contribute a considerable proportion of AA to swine diets (Sauer et al., 2001), although their CP and AA concentrations are quite low. As discussed by Fan et al. (1994), the relationship between dietary AA content and AID value of respective AA is quadratic with plateau, and the plateau AID could be determined by meeting the threshold level of AA to become independent of

the dietary AA content. When the direct method is used, the determination of AID of CP and AA in a cereal grain is generally underestimated because it is difficult to meet the threshold level of CP or AA in the diet with a cereal grain as the sole source of CP or AA, and therefore underestimates the AID value in the mixed diet containing the cereal grain (Fan et al., 1994; Stein et al., 2005). This hypothesis was supported by Stein et al. (2005) and Xue et al. (2014), where the mixed diets containing corn resulted in lack of additivity. However, the AID of CP and most indispensable AA in the wheat-CM-MBM diet was additive regardless of the choice of DIM in this study. The difference of additivity between corn and wheat might be explained by the greater CP and AA contents in wheat than corn, which can elicit a relatively large change in the determination of AID values (Fan et al., 1994). Furthermore, it has been reported that direct method did not underestimate the AID of AA in wheat compared with difference and regression methods (Hennig et al., 2008), which supports the use of wheat as a sole source of nitrogen to determine AID of AA.

The wheat-CM-MBM and wheat-MBM-DDGS diets contained the same levels of wheat and MBM to elucidate whether there was a different effect between CM and DDGS on additivity of AID of CP and AA determined with the same DIM. In the wheat-MBM-DDGS diet, AID of CP and four indispensable AA using TiO_2 as DIM, and of CP and seven indispensable AA using Cr_2O_3 as DIM were underestimated. The lack of additivity observed with TiO_2 and Cr_2O_3 in the wheat-MBM-DDGS diet might relate to the low recovery of DIM in the presence of high concentrations of fiber from DDGS contained in this diet. It has been reported that the recovery of TiO_2 was decreased from 99.9% to 67.8% and the recovery of Cr_2O_3 decreased from 84.9% to 60.6% with increasing dietary NDF concentration using pigs installed with post-valve T-caecum cannulas (Yin et al., 2000). Furthermore, a negative correlation between NDF and AID value of CP and AA

determined with Cr_2O_3 has been reported (Fan et al., 2001); however, further research is required to investigate whether similarly negative correlation exists when TiO_2 is chosen as DIM.

Unlike in the mixed diets containing three feed ingredients, the AID of CP and indispensable AA were not additive in the wheat-CM-MBM-DDGS diet containing four feed ingredients using either Cr_2O_3 or TiO_2 as DIM. Except for dietary AA content, fiber source and level can also affect the threshold AA level in determination of AID of AA in ingredients, and therefore affect the predicted AID in mixed diets (Fan et al., 1994; Xue et al., 2014). However, the acid and neutral detergent fiber contents in the wheat-CM-MBM-DDGS diet were between those in the other two mixed diets, which indicates fiber might not be the reason for the lack of additivity of AID of CP and AA in the wheat-CM-MBM-DDGS diet. On the other hand, four components were included in the determination of predicted AID values in the wheat-CM-MBM-DDGS diet, and this cumulative calculation could magnify the error in the mixed diet induced by the calculation of determining AID for ingredients (Sauer et al., 2001); however, this speculation was not supported by the data in the study reported by Xue et al. (2014), and further study is required.

5.6 Conclusion

In conclusion, the SID of CP and AA are generally more accurate than AID for predicting ileal digestibility of CP and AA in mixed diets containing wheat, canola meal, meat and bone meal, and sorghum distillers' dried grains with solubles. The results of the experiment also demonstrate that the determination of endogenous loss, AID, and SID of CP and AA was not affected by the choice of DIM (Cr_2O_3 or TiO_2). In addition, the choice of DIM has no effect on the additivity of AID and SID of CP and most indispensable AA in all the three mixed diets.

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Table 5-1. Ingredient composition of experimental diets, % as-fed basis[1]

Item	NFD	Wheat	CM	MBM	DDGS	Wheat-CM-MBM	Wheat-MBM-DDGS	Wheat-CM-MBM-DDGS
Wheat	-	88.90	-	-	-	58.00	58.00	58.00
CM	-	-	42.50	-	-	17.80	-	10.50
MBM	-	-	-	27.00	-	6.00	6.00	6.00
DDGS	-	-	-	-	58.50	-	24.40	10.00
Corn starch	72.40	-	47.50	64.40	30.90	9.60	3.00	6.90
Dextrose	10.00	-	-	-	-	-	-	-
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Chromic oxide premix[2]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Titanium dioxide premix[3]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Monocalcium phosphate	2.80	1.50	0.70	-	0.70	-	-	-
Limestone	0.60	1.00	0.70	-	1.30	-	-	-
Cellulose[4]	5.00	-	-	-	-	-	-	-
Salt	0.40	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Potassium carbonate	0.40	-	-	-	-	-	-	-
Magnesium oxide	0.10	-	-	-	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]NFD = nitrogen-free diet; CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles.

[2]A 5 g/kg chromic oxide was added to 20 g corn starch.

[3]A 5 g/kg titanium dioxide was added to 20 g corn starch.

[4]Solka-Floc 40 FCC, International Fiber Corporation, Urbana, OH.

[5]Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D_3, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; $_D$-pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B_{12}, 0.02 mg.

[6]Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

[7]Provided 0.3 mg Se/kg of complete diet.

Table 5-2. Analyzed nutrient composition in ingredients, % as-fed basis[1]

Item	Wheat	Canola meal	Meat and bone meal[2]	DDGS[1]
Dry matter	88.55	90.49	92.94	86.32
Crude protein[3]	11.19	40.24	60.31	29.82
Acid detergent fiber	3.41	19.12	5.43	9.38
Neutral detergent fiber	16.09	25.49	35.49	27.97
Ether extract	0.65	5.83	8.54	10.80
Indispensable amino acid				
Arg	0.51	2.14	3.75	1.10
His	0.25	1.00	0.96	0.54
Ile	0.40	1.62	1.96	1.33
Leu	0.70	2.62	3.58	3.15
Lys	0.36	2.03	2.73	0.72
Met	0.16	0.73	0.78	0.43
Phe	0.47	1.55	2.10	1.46
Thr	0.30	1.53	1.82	1.02
Trp	0.14	0.47	0.32	0.19
Val	0.49	2.05	2.79	1.57
Dispensable amino acid				
Ala	0.40	1.61	3.93	2.12
Asp	0.55	2.60	4.06	1.88
Cys	0.25	0.95	0.79	0.48
Glu	2.77	6.23	6.50	3.77
Gly	0.47	1.85	6.77	1.03
Pro	0.94	2.39	4.61	1.83
Ser	0.41	1.23	2.19	1.07
Tyr	0.26	1.02	1.47	1.05

[1]DDGS = sorghum distillers' dried grains with solubles.
[2]Acid and neutral detergent fiber in meat and bone meal probably represent gelatin and other components dissolved in the acid and neutral detergent solutions rather than fiber.
[3]Crude protein = nitrogen × 6.25.

Table 5-3. Analyzed nutrient composition in experimental diets, % as-fed basis[1]

Item	NFD	Wheat	CM	MBM	DDGS	Wheat-CM-MBM	Wheat-MBM-DDGS	Wheat-CM-MBM-DDGS
Dry matter	87.88	87.72	88.18	90.82	87.52	88.02	87.92	87.85
Crude protein[2]	0.68	9.79	16.95	17.72	16.86	16.85	17.20	17.44
Indispensable amino acid								
Arg	0.01	0.47	0.96	1.08	0.62	0.97	0.74	0.84
His	0.00	0.21	0.44	0.25	0.31	0.40	0.33	0.36
Ile	0.02	0.37	0.73	0.59	0.74	0.69	0.64	0.65
Leu	0.04	0.65	1.20	1.09	1.78	1.18	1.33	1.21
Lys	0.01	0.29	0.91	0.81	0.41	0.78	0.53	0.64
Met	0.01	0.14	0.32	0.22	0.25	0.28	0.24	0.25
Phe	0.02	0.45	0.70	0.64	0.82	0.72	0.72	0.71
Thr	0.01	0.27	0.70	0.53	0.58	0.59	0.50	0.53
Trp	0.02	0.11	0.20	0.10	0.12	0.19	0.15	0.17
Val	0.01	0.44	0.90	0.85	0.89	0.86	0.78	0.80
Dispensable amino acid								
Ala	0.02	0.36	0.75	1.21	1.23	0.82	0.94	0.85
Asp	0.02	0.50	1.18	1.20	1.13	1.11	1.01	1.02
Cys	0.01	0.21	0.42	0.23	0.29	0.40	0.28	0.36
Glu	0.04	2.56	2.89	1.98	2.43	3.32	2.94	3.11
Gly	0.02	0.44	0.88	2.07	0.61	1.09	0.90	1.01
Pro	0.02	0.92	1.06	1.41	1.09	1.29	1.22	1.24
Ser	0.01	0.36	0.59	0.61	0.58	0.59	0.56	0.57
Tyr	0.01	0.29	0.44	0.40	0.54	0.45	0.47	0.45

[1]NFD = nitrogen-free diet; CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles.

[2]Crude protein = nitrogen × 6.25.

Table 5-4. Basal ileal endogenous losses of crude protein (CP) and amino acids (AA) in pigs fed nitrogen-free diet, mg/kg dry matter intake[1,2]

Item	Cr_2O_3	TiO_2	SEM	*P*-value
CP, g/kg dry matter intake[2]	16.31	16.91	1.708	0.170
Indispensable AA				
Arg	503	520	71.2	0.203
His	181	188	13.7	0.159
Ile	368	383	28.4	0.148
Leu	587	611	40.8	0.132
Lys	741	774	75.6	0.149
Met	91	95	10.5	0.182
Phe	361	375	24.0	0.139
Thr	540	560	43.7	0.136
Trp	114	119	9.3	0.108
Val	531	551	39.3	0.134
Dispensable AA				
Ala	576	598	50.0	0.162
Asp	856	889	61.3	0.142
Cys	179	185	12.5	0.138
Glu	1,017	1,057	85.4	0.147
Gly	1,241	1,285	186.6	0.213
Pro	3,549	3,672	871.1	0.274
Ser	466	483	41.5	0.154
Tyr	276	286	18.0	0.145

[1] Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.
[2]Each least squares mean represents eight observations.
[3]Crude protein = nitrogen × 6.25.

Table 5-5. Measured apparent ileal digestibility (%) of crude protein (CP), dry matter (DM), and amino acids (AA) in ingredients and mixed diets[1,2]

	Wheat		CM		MBM		DDGS		Wheat-CM-MBM		Wheat-MBM-DDGS		Wheat-CM-MBM-DDGS		SEM[3]	*P*-value		
Item	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2		Diet	Marker	Diet × Marker
CP	72.2	70.8	67.6	67.9	59.5	58.7	52.0	52.7	69.7	70.0	66.5	65.5	69.2	69.6	1.51	<0.001	0.684	0.434
DM	79.5	78.3	69.5	69.8	81.0	80.7	62.0	62.5	73.3	73.5	72.2	71.3	72.3	72.6	0.97	<0.001	0.771	0.383
Indispensable AA																		
Arg	81.7	80.7	83.9	84.0	74.7	74.2	64.7	65.2	82.3	82.4	76.6	75.9	79.8	80.0	1.16	<0.001	0.627	0.421
His	80.9	79.8	80.3	80.5	54.1	53.2	55.3	55.9	79.0	79.1	70.7	69.8	75.4	75.7	1.35	<0.001	0.579	0.513
Ile	79.0	77.9	75.8	76.0	63.9	63.2	65.0	65.5	76.8	77.0	72.3	71.5	75.5	75.8	1.33	<0.001	0.623	0.412
Leu	81.0	80.0	78.2	78.4	65.1	64.4	69.5	70.0	78.2	78.3	75.1	74.3	77.2	77.5	1.49	<0.001	0.619	0.433
Lys	48.5	45.9	70.5	70.7	48.2	47.4	17.0	17.9	65.9	66.1	47.0	45.4	58.7	59.2	2.63	<0.001	0.602	0.357
Met	81.5	80.4	84.7	84.8	61.9	61.1	67.3	67.7	81.0	81.1	74.1	73.3	78.6	78.8	1.27	<0.001	0.566	0.428
Phe	82.9	82.0	78.2	78.4	67.2	66.5	67.6	68.1	79.1	79.3	75.3	74.6	78.2	78.4	1.30	<0.001	0.650	0.452
Thr	63.7	61.8	70.0	70.3	54.7	53.7	55.8	56.4	67.7	68.0	61.3	60.1	65.4	65.8	1.87	<0.001	0.573	0.337
Trp	81.3	80.3	87.6	87.7	68.8	68.2	75.2	75.5	85.1	85.2	81.9	81.3	83.7	83.9	1.15	<0.001	0.507	0.360
Val	74.6	73.3	72.5	72.8	63.9	63.1	61.8	62.3	73.3	73.5	69.0	68.0	71.8	72.1	1.43	<0.001	0.620	0.389
Dispensable AA																		
Ala	66.0	64.3	74.0	74.3	70.9	70.2	65.6	66.1	72.0	72.2	68.8	67.9	70.9	71.2	1.53	0.001	0.596	0.284
Asp	65.5	63.8	71.0	71.3	45.4	44.2	55.1	55.7	66.1	66.3	58.3	57.0	62.2	62.6	1.87	<0.001	0.525	0.369
Cys	81.3	80.4	74.1	74.3	44.0	42.8	55.6	56.2	75.1	75.3	65.6	64.6	74.2	74.5	1.81	<0.001	0.554	0.461
Glu	91.6	91.1	84.0	84.1	62.6	61.8	64.2	64.7	85.7	85.8	80.1	79.6	83.8	84.0	1.32	<0.001	0.674	0.586
Gly	67.2	65.5	68.3	68.5	71.3	70.8	40.4	41.1	70.2	70.3	63.4	62.4	68.2	68.6	2.11	<0.001	0.677	0.402
Pro	76.5	75.5	62.4	62.5	59.2	58.8	36.2	37.0	72.6	72.8	66.0	65.4	67.2	67.6	5.38	<0.001	0.921	0.658
Ser	78.9	77.8	72.1	72.4	58.7	57.7	58.6	59.2	72.4	72.6	68.8	67.9	71.2	71.6	1.80	<0.001	0.641	0.434
Tyr	81.8	80.9	77.3	77.5	60.2	59.5	68.4	68.8	77.6	77.8	75.1	74.3	77.3	77.5	1.37	<0.001	0.625	0.473

[1]CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]Each least squares mean represents 8 observations.

[3]SEM of diet.

Table 5-6. Measured standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in ingredients and mixed diets[1,2]

	Wheat		CM		MBM		DDGS		Wheat-CM-MBM		Wheat-MBM-DDGS		Wheat-CM-MBM-DDGS		SEM[3]	*P*-value		
Item	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2		Diet	Marker	Diet × Marker
CP	86.8	86.0	76.1	76.7	67.8	67.4	60.5	61.5	78.3	78.8	74.8	74.2	77.5	78.1	1.51	<0.001	0.824	0.515
Indispensable AA																		
Arg	91.0	90.4	88.5	88.8	79.0	78.6	71.8	72.5	86.9	87.1	82.5	82.1	85.0	85.4	1.16	<0.001	0.954	0.473
His	88.4	87.7	83.9	84.2	60.7	60.0	60.4	61.2	82.9	83.2	75.5	74.8	79.8	80.2	1.35	<0.001	0.914	0.577
Ile	87.7	87.0	80.3	80.7	69.6	69.1	69.4	70.1	81.5	81.8	77.4	76.7	80.5	81.0	1.33	<0.001	0.993	0.500
Leu	88.9	88.3	82.5	82.9	70.0	69.5	72.4	73.0	82.6	82.9	79.0	78.4	81.5	81.9	1.49	<0.001	0.977	0.522
Lys	70.9	69.3	77.7	78.2	56.5	56.1	32.8	34.5	74.2	74.8	59.3	58.3	68.8	69.8	2.63	<0.001	0.897	0.477
Met	87.2	86.4	87.2	87.4	65.6	65.0	70.5	71.1	83.9	84.1	77.4	76.7	81.8	82.1	1.27	<0.001	0.814	0.489
Phe	90.0	89.3	82.7	83.1	72.3	71.9	71.5	72.1	83.5	83.8	79.7	79.2	82.7	83.1	1.30	<0.001	0.968	0.515
Thr	81.2	80.0	76.8	77.3	63.9	63.3	64.0	64.9	75.8	76.3	70.8	70.0	74.3	75.1	1.87	<0.001	0.992	0.471
Trp	90.4	89.7	92.6	92.9	79.2	79.0	83.5	84.2	90.3	90.7	88.6	88.3	89.6	90.1	1.15	<0.001	0.729	0.476
Val	85.2	84.3	77.8	78.2	69.6	69.0	67.0	67.7	78.7	79.1	74.9	74.2	77.6	78.1	1.43	<0.001	0.987	0.477
Dispensable AA																		
Ala	80.0	78.8	80.8	81.3	75.2	74.7	69.7	70.3	78.2	78.7	74.2	73.5	76.8	77.4	1.53	<0.001	0.956	0.409
Asp	80.5	79.3	77.4	77.9	51.8	50.9	61.7	62.6	72.9	73.3	65.8	64.7	69.6	70.3	1.87	<0.001	0.890	0.458
Cys	88.8	88.1	77.9	78.2	51.1	50.1	61.0	61.8	79.1	79.3	71.2	70.4	78.6	79.0	1.81	<0.001	0.862	0.528
Glu	95.1	94.8	87.1	87.3	67.3	66.7	67.8	68.5	88.4	88.6	83.2	82.7	86.7	86.9	1.32	<0.001	0.985	0.614
Gly	91.9	91.1	80.7	81.3	76.8	76.4	58.2	59.6	80.2	80.7	75.5	74.9	79.0	79.7	2.11	<0.001	0.713	0.485
Pro	110.4	110.6	91.9	93.1	82.0	82.4	64.7	66.5	96.8	97.8	91.5	91.8	92.3	93.6	5.38	<0.001	0.173	0.691
Ser	90.3	89.6	79.1	79.6	65.7	64.9	65.6	66.5	79.4	79.8	76.2	75.5	78.4	79.0	1.80	<0.001	0.933	0.483
Tyr	90.2	89.5	82.8	83.2	66.5	66.0	72.8	73.4	83.0	83.4	80.2	79.7	82.7	83.1	1.37	<0.001	0.934	0.543

[1]CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]Each least squares mean represents 8 observations.

[3]SEM of diet.

Table 5-7. Differences between measured and predicted values for apparent ileal digestibility (AID, %) of crude protein (CP) and amino acids (AA) in mixed diets[1,2]

	Wheat-CM-MBM				Wheat-MBM-DDGS				Wheat-CM-MBM-DDGS			
	Cr_2O_3		TiO_2		Cr_2O_3		TiO_2		Cr_2O_3		TiO_2	
Item	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM
CP	1.9	1.15	2.7	1.27	5.6**	1.38	5.0**	1.25	3.5*	1.36	4.4*	1.55
Indispensable AA												
Arg	1.3	0.73	1.8	0.82	2.7*	1.00	2.4*	0.87	0.6	1.13	1.2	1.26
His	2.3*	0.86	2.9*	0.99	4.4**	1.09	3.9*	1.14	2.5*	0.84	3.2*	1.14
Ile	2.0	0.99	2.5	1.10	2.5*	0.86	1.9	0.90	2.9*	0.91	3.5*	1.09
Leu	1.4	0.92	2.0	1.02	2.7*	1.04	2.1	0.93	2.6*	0.93	3.1*	1.14
Lys	7.3**	1.35	8.4**	1.49	10.0*	2.88	9.3*	3.00	5.5*	1.97	6.8*	2.13
Met	1.3	0.84	1.9	0.89	2.3*	0.85	1.8	0.86	2.0*	0.77	2.6*	0.91
Phe	0.9	0.91	1.5	0.98	2.0	0.90	1.5	0.83	2.3*	0.79	2.8*	0.98
Thr	2.7	1.29	3.6*	1.48	3.2	1.45	2.5	1.44	3.1*	0.98	4.0*	1.30
Trp	2.2*	0.84	2.8*	0.86	4.2**	0.88	4.1**	1.02	2.6**	0.67	3.3**	0.82
Val	1.7	1.05	2.4	1.18	2.3	1.12	1.7	1.02	2.2	0.99	3.0*	1.25
Dispensable AA												
Ala	1.4	1.11	2.3	1.24	1.8	1.26	1.1	1.08	1.9	1.15	2.7	1.35
Asp	2.9	1.25	3.8*	1.50	2.3	1.79	1.5	1.99	1.5	1.15	2.5	1.54
Cys	2.2	1.17	2.8	1.34	-0.2	1.27	-0.9	1.34	3.5**	0.72	4.3**	0.93
Glu	0.5	0.55	0.8	0.65	1.0	0.85	0.5	0.79	0.9	0.65	1.3	0.80
Gly	-0.2	1.14	0.6	1.47	2.0	1.90	1.5	1.81	-1.4	2.08	-0.5	2.19
Pro	4.9	2.25	5.5*	2.25	12.6**	3.59	12.2**	3.34	-2.5	7.25	-1.7	7.26
Ser	0.4	1.10	1.2	1.22	2.5	1.46	1.9	1.41	1.3	1.03	2.1	1.28
Tyr	2.3*	0.83	2.8*	0.95	4.0**	0.91	3.5**	0.87	3.9**	0.72	4.4**	0.92

[1]Difference = Measured – Predicted AID of CP and AA; predicted AID was determined based on the measured AID values in the feed ingredients contained in mixed diet.

[2]CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; Diff = difference.

*P < 0.05; **P < 0.01. Ileal digestibility was underestimated when the difference between measured and predicted ileal digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

Table 5-8. Differences between measured and predicted values for standardized ileal digestibility (SID, %) of crude protein (CP) and amino acids (AA) in mixed diets[1,2]

	Wheat-CM-MBM				Wheat-MBM-DDGS				Wheat-CM-MBM-DDGS			
	Cr_2O_3		TiO_2		Cr_2O_3		TiO_2		Cr_2O_3		TiO_2	
Item	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM	Diff	SEM
CP	-0.4	1.15	0.3	1.27	3.2	1.38	2.5	1.25	1.0	1.36	1.7	1.55
Indispensable AA												
Arg	-0.2	0.73	0.2	0.82	1.6	1.00	1.2	0.87	-0.7	1.13	-0.1	1.26
His	0.7	0.86	1.2	0.99	2.8*	1.09	2.2	1.14	1.0	0.84	1.6	1.14
Ile	0.4	0.99	0.9	1.10	1.5	0.86	0.9	0.90	1.7	0.91	2.2	1.09
Leu	0.0	0.92	0.5	1.02	1.9	1.04	1.3	0.93	1.6	0.93	2.1	1.14
Lys	3.9*	1.35	4.8*	1.49	6.2	2.88	5.3	3.00	2.4	1.97	3.6	2.13
Met	0.4	0.84	0.9	0.89	1.4	0.85	0.9	0.86	1.2	0.77	1.8	0.91
Phe	-0.3	0.91	0.2	0.98	1.2	0.90	0.7	0.83	1.3	0.79	1.8	0.98
Thr	0.1	1.29	0.9	1.48	1.2	1.45	0.5	1.44	1.0	0.98	1.9	1.30
Trp	0.1	0.84	0.6	0.86	1.9	0.88	1.7	1.02	0.6	0.67	1.1	0.82
Val	-0.1	1.05	0.6	1.18	1.1	1.12	0.5	1.02	0.9	0.99	1.6	1.25
Dispensable AA												
Ala	-0.6	1.11	0.1	1.24	0.7	1.26	0.0	1.08	0.5	1.15	1.2	1.35
Asp	0.6	1.25	1.4	1.50	0.5	1.79	-0.3	1.99	-0.3	1.15	0.7	1.54
Cys	0.5	1.17	1.0	1.34	-1.2	1.27	-1.9	1.34	1.9*	0.72	2.5*	0.93
Glu	-0.3	0.55	0.0	0.65	0.3	0.85	-0.1	0.79	0.2	0.65	0.6	0.80
Gly	-3.1*	1.14	-2.5	1.47	-0.3	1.90	-0.9	1.81	-4.2	2.08	-3.3	2.19
Pro	-0.9	2.25	-0.4	2.25	8.6*	3.59	8.1*	3.34	-7.2	7.25	-6.5	7.26
Ser	-1.4	1.10	-0.7	1.22	1.2	1.46	0.5	1.41	-0.2	1.03	0.5	1.28
Tyr	1.0	0.83	1.5	0.95	3.2**	0.91	2.6*	0.87	2.9**	0.72	3.4**	0.92

[1]Difference = Measured – Predicted SID of CP and AA; predicted SID was determined based on the measured SID values in the feed ingredients contained in mixed diet.

[2]CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide; Diff = difference.

*P < 0.05; **P < 0.01. Ileal digestibility was underestimated when the difference between measured and predicted ileal digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

CHAPTER 6. INVESTIGATION OF XYLANASE, DIET FORMULATION METHOD FOR ENERGY, AND CHOICE OF DIGESTIBILITY INDEX MARKER ON NUTRIENT AND ENERGY UTILIZATION FOR BROILER CHICKENS AND PIGS

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6.1 Abstract

The aim of this study was to investigate the growth performance and nutrient utilization responses of broiler chickens and the nutrient utilization of pigs to xylanase, experimental diet formulation method for energy (FME), and digestibility index marker (DIM). In Exp. 1, a total of 448 male broiler chickens were used in a randomized complete block design with BW as a blocking factor. Seven dietary treatments were prepared in a 3 × 2 + 1 factorial arrangement with inclusion of sand, diatomaceous earth (DAE), or wheat bran (WB) as FME and without or with xylanase (26,400 unit/kg of diet) plus positive control. Each of chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) were incorporated at 5 g/kg in diets. There were 8 replicate cages of 8 birds per cage. Birds were weighed on d 7, 14, and 21, and feed intake was recorded. Excreta samples were collected from d 19 to 21. On d 21, birds were euthanized, and ileal digesta samples were collected. In Exp. 2, twenty-one barrows (initial BW = 33.0 ± 0.3 kg), fitted with simple T-cannulas at the distal ileum, were used in a triplicate 7 × 2 incomplete Latin Square design with 7 dietary treatments, which were prepared by the same arrangement as in broiler chickens, and 2 periods giving 6 replicates per diet. Fecal samples were collected on d 4 and 5, and ileal digesta samples were collected on d 6 and 7 of each period. In Exp. 1, the growth performance of birds was not affected by xylanase but was affected by the choice of FME. There were interactions ($P < 0.05$) between

xylanase and FME for apparent ileal digestibility (AID) of crude protein, His, Met, Thr, and Trp. Inclusion of xylanase decreased ($P < 0.05$) the AID of crude protein and Trp in the sand diet and AID of His and Thr in the DAE diet, but increased ($P < 0.05$) AID of Met in the WB diet. The AID of energy and nutrients except Arg and Met were greater ($P < 0.05$) observed with Cr_2O_3 than TiO_2 as DIM. In Exp. 2, there were interactions ($P < 0.05$) between xylanase and FME for AID of dry matter, energy, Arg, and Lys. Inclusion of xylanase decreased ($P < 0.05$) the AID of Lys in the DAE or WB diet, but increased ($P < 0.05$) AID of Arg in the sand diet and AID of energy in the WB diet. The DIM type had no effect on responses in pigs. In conclusion, the efficacy of xylanase on ileal energy and amino acid digestibility depends on the choice of formulation method for energy in broiler chickens and pigs, and DIM affects the determination of ileal digestibility in broiler chickens.

Key words: chromic oxide, digestibility, poultry, swine, titanium dioxide, xylanase

6.2 Introduction

Xylanase is one of the exogenous enzymes that has been widely used for commercial feeds in the poultry and swine industry (Adeola and Cowieson, 2011). The evaluation of xylanase has been conducted in wheat-based diets (Yin et al., 2000b; Cowieson and Masey O'Neill, 2013) and corn-soybean meal-based (CSBM) diets (Stefanello et al., 2016; Cho et al., 2017) in both chickens and pigs. Negative control diets formulated to contain less energy than positive control (PC) diets are commonly used to evaluate the efficacy of xylanase supplementation (Liu and Fowler, 2017). To replace part of the energy-contributing components, wheat bran (WB), sand, or diatomaceous earth (DAE) have been used in previous studies (Karimi et al., 2013; Liu and Fowler, 2017). However, whether the experimental diet formulation method for energy (FME) reduction of the

test diet achieved by including WB, sand, or DAE affects the evaluation of xylanase on growth performance or efficiency of nutrient utilization in chickens or pigs is still unknown.

The interaction between dietary fiber and digestibility index marker (DIM) type has been reported in previous studies (Yin et al., 2000b; Wang et al., 2017). The main function of xylanase is to hydrolyze dietary fiber (Adeola and Cowieson, 2011), which might interfere with the interaction between fiber and DIM, and it is reasonable to speculate that the effect of FME and xylanase may depend on the choice of DIM. Therefore, the objective of the present study was to evaluate the effect of inclusion of xylanase, FME, and DIM on growth performance in broiler chickens and nutrient and energy utilization in both broiler chickens and pigs.

6.3 Materials and Methods

All animal procedures used in this study were approved by the Purdue University Animal Care and Use Committee.

Seven diets were prepared in a 3 × 2 + 1 factorial arrangement with 50 g/kg sand, 50 g/kg DAE, or 75 g/kg WB as FME and without or with xylanase plus PC for chickens and pigs, respectively (Table 6-1). The xylanase activity was 26,400 unit/kg of complete diet. All diets were formulated to meet or exceed the vitamin and mineral requirement estimates for both broiler chickens (NRC, 1994) and pigs (NRC, 2012). Both chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) were added to experimental diets as the DIM at 5 g/kg of diet, respectively.

Exp. 1: Growth Performance and Nutrient and Energy Utilization in Broiler Chickens

A total of 448 male broiler chicks (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR) were obtained from a commercial hatchery. On d 0, tagged birds were individually weighed and assigned to 7 experimental diets in a randomized complete block design with BW as a blocking

factor. There were 8 replicate cages for each treatment and 8 birds were housed in each cage. Birds had free access to feed and water during the experimental period. Birds were raised in electrically heated battery brooders (model SB 4 T, Alternative Design Manufacturing, Siloam Springs, AR), which maintained the temperature at 35, 31, and 27°C from d 0 to 7, d 7 to 14, and d 14 to 21, respectively. On d 7, 14, and 21, all birds were weighed individually, and feed consumption was recorded for each cage. On d 19, 20, and 21, uncontaminated excreta samples were collected daily. On d 21, birds were euthanized by CO_2 asphyxiation and dissected for the collection of ileal digesta samples from the distal two-thirds of the ileum. Both excreta samples and ileal digesta samples were pooled within cages and stored at –20°C until further analyses.

Exp. 2: Apparent Nutrient and Energy Utilization in Pigs

Twenty-one barrows (initial BW = 33.0 ± 0.3 kg) surgically fitted with simple T-cannulas at the distal ileum according to the description of Dilger et al. (2004) were used. Pigs were allotted to 3 blocks based on BW and assigned to a triplicate 7 × 2 incomplete Latin Square design with 7 experimental diets and 2 periods, which provided 6 observations for each treatment. All pigs were individually housed in stainless steel crates (1.22 × 1.22 m) equipped with a feeder and a nipple drinker. Daily feed allowance was calculated as 4% of the initial smallest BW in each block and period, and pigs were fed at 0800 and 1700 h in 2 equal meals, and water was available all the time. Each experimental period consisted of a 3-d adaptation, 2-d feces collection, and 2-d ileal digesta collection. On d 4 and 5, representative fecal samples were collected after the meal in the morning. On d 6 and 7, Whirl-Pak® bag (Nasco, Fort Atkinson, WI) containing 10 mL of 10% formic acid was attached to the T-cannula to collect ileal digesta from 0800 to 1700 h. Attached bags were inspected every 30 min, and the filled bags were changed and stored at –20°C

immediately. After each experimental period, both ileal digesta samples and fecal samples were pooled within periods and pigs, and subsamples were stored at –20°C until further processing.

6.3.1 Chemical Analysis and Calculation

Ileal digesta samples from Exp. 1 and 2 were lyophilized, and excreta samples from Exp. 1 and fecal samples from Exp. 2 were forced-air dried at 55°C to constant weight. Diet, excreta, and fecal samples were ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany) and ileal digesta samples were ground in a coffee grinder. The concentration of dry matter (DM) in diets, freeze-dried ileal digesta, and oven-dried excreta and feces samples were determined by drying in a forced-air oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Nitrogen content in diets and ileal digesta were determined with the combustion method (TruMac® N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the crude protein (CP) content was calculated as N × 6.25. Gross energy (GE) content was analyzed by isoperibol bomb calorimetry using a Parr 6200 calorimeter (PARR Instrument Co., Moline, IL). The acid detergent fiber [method 973.18 (AD); AOAC, 2006] and neutral detergent fiber (Van Soest et al., 1991) contents in diets were analyzed with a fiber analyzer (ANKOM A2000 Fiber Analyzer; ANKOM Technology, Macedon, NY). The contents of amino acids (AA) in diets and ileal digesta samples were determined by HPLC [method 982.30 E (a, b, c); AOAC, 2006], which was performed by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). The concentrations of Cr and Ti were determined as described by Saha and Gilbreath (1991) and Myers et al. (2004), respectively with few modifications as indicated by Wang and Adeola (2018).

The apparent ileal digestibility (AID), apparent total tract digestibility (ATTD) for pigs, and apparent total tract utilization (ATTU) for chickens of GE were calculated using the following equations (Adeola, 2001):

$$AID, \% = [1 - (DIM_{diet}/DIM_{ileal}) \times (GE_{ileal}/GE_{diet})] \times 100;$$

$$ATTD, \% = [1 - (DIM_{diet}/DIM_{feces}) \times (GE_{feces}/GE_{diet})] \times 100;$$

$$ATTU, \% = [1 - (DIM_{diet}/DIM_{excreta}) \times (GE_{excreta}/GE_{diet})] \times 100;$$

where DIM_{diet}, DIM_{ileal}, DIM_{feces}, and $DIM_{excreta}$ are the DIM concentration in diet, ileal digesta, feces, and excreta, respectively (mg/kg DM); GE_{diet}, GE_{ileal}, GE_{feces}, and $GE_{excreta}$ are the GE concentrations (kcal/kg DM) of diet, ileal digesta, feces, and excreta, respectively. The AID of DM, CP, or AA was calculated with GE replaced by DM, CP, or AA (mg/kg DM), and the ATTD or ATTU of DM was calculated with GE replaced by DM (mg/kg).

6.3.2 Statistical Analysis

The data on growth performance in broiler chickens was analyzed using GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). In the model, fixed effects were control and factors nested in control, which included xylanase, FME, and the interaction between xylanase and FME; and random effect was block.

The data on energy and nutrient utilization in both broiler chickens and pigs were analyzed using GLIMMIX procedure of SAS as a split-plot arrangement, with dietary treatment as whole-plot factor and the DIM type as split-plot factor. For broiler chickens, fixed effects were control and factors nested in control, which included xylanase, FME, interaction between them, and DIM; and random effects were block and factors nested in control, which included 2-way interaction of block and DIM, 3-way interaction of block, DIM, and xylanase, 3-way interaction of block, DIM, and FME, and 4-way interaction of block, DIM, xylanase, and FME. In the model used in pigs,

the fixed effects were the same with those in the broiler chicken study, and the random effects were period, replication, pig, and factors nested in control, which included 2-way interaction of period and DIM, 3-way interaction of replication, FME, and DIM, and 3-way interaction of pig, FME, and DIM.

Least squares means were calculated and separated by the SLICEDIFF option with Bonferroni's correction when there was interaction between FME and xylanase, or separated by the PDIFF option with Bonferroni's correction when there was no interaction between them. The experimental unit was cage in Exp. 1 and pig in Exp. 2 and statistically significant difference was set at $P \leq 0.05$.

6.4 Results

In Exp. 1, the PC treatment had greater ($P < 0.001$) BW gain from d 0 to 7, and greater G:F ($P < 0.001$) during the whole experimental period compared with other treatments as shown in Table 6-2. There was no interaction between xylanase and FME and the inclusion of xylanase did not affect the growth performance of broiler chickens during the whole experimental period. During d 0 to 7, however, the chickens fed the diet containing DAE had lower ($P < 0.05$) BW gain and G:F compared with the chickens fed the diet containing WB. During d 14 to 21 and d 0 to 21, the chickens fed the diet containing DAE had lower ($P < 0.05$) BW gain compared with the chickens fed the diet containing sand and lower ($P < 0.05$) feed intake (**FI**) compared with the chickens fed the diet containing WB.

Compared with other treatments, the PC treatment had greater ($P < 0.05$) AID of DM and GE, but similar AID of CP and ATTU of DM and GE (Table 6-3). There was an interaction ($P < 0.05$) between xylanase and FME for AID of CP and the inclusion of xylanase in the diet containing

sand decreased ($P < 0.001$) the AID of CP but had no effect when alternate FME were used. There was no effect of inclusion of xylanase on AID and ATTU of DM and GE. The chickens fed the diet containing WB had greater ($P < 0.05$) AID of DM compared with the chickens fed the diet containing DAE and greater ($P < 0.05$) ATTU of DM compared with the chickens fed the diet containing sand. As for the digestibility of energy, the chickens fed the diet containing WB had the lowest ($P < 0.05$) AID of GE, but greatest ($P < 0.05$) ATTU of GE compared with the chickens fed the diet containing DAE or sand. The AID of DM, CP, and GE observed with Cr_2O_3 as marker observed with either Cr_2O_3 or TiO_2 as marker.

Except for the AID of Trp ($P < 0.05$), there were no differences between the PC and other treatments for AID of AA in broiler chickens (Table 6-4). There were interactions ($P < 0.05$) between xylanase and FME for AID of His, Met, Thr, and Trp. Inclusion of xylanase decreased ($P < 0.05$) AID of Trp in the diet containing sand and decreased ($P < 0.05$) AID of His and Thr in the diet containing DAE, but increased ($P < 0.05$) AID of Met in the diet containing WB. The AID of Arg, Ile, Leu, Lys, Phe, and Val were not affected by the inclusion of xylanase in diet, but were affected by FME, where the diet containing WB had lower ($P < 0.05$) AID values compared with the diet containing either sand or DAE. The AID of all AA except Arg and Met was greater ($P < 0.05$) observed with Cr_2O_3 compared with TiO_2 as DIM.

In Exp. 2, the PC treatment had greater ($P < 0.05$) ATTD of DM compared with other treatments as shown in Tables 6-5. There were interactions ($P < 0.05$) between xylanase and FME for AID of DM and GE. Inclusion of xylanase decreased ($P < 0.05$) AID of DM in the diet containing sand, however, increased ($P < 0.05$) AID of GE in the diet containing WB. The ATTD of DM in the diet containing WB was greater ($P < 0.05$) than that in the diet containing sand or DAE, oppositely, the ATTD of GE in the diet containing WB was lower ($P < 0.05$) than that in the

diet containing either sand or DAE. The choice of DIM had no effect on the determination of AID of DM, CP, and GE and ATTD of DM and GE.

There were interactions ($P < 0.05$) between xylanase and FME for AID of Arg and Lys (Table 6-6). Inclusion of xylanase decreased ($P < 0.05$) AID of Arg and Lys in the diet containing DAE and decreased ($P < 0.05$) AID of Lys in the diet containing WB, but increased ($P < 0.05$) AID of Arg in the diet containing sand. Regardless of FME, xylanase decreased ($P < 0.05$) AID of Ile and Met but increased ($P < 0.05$) AID of Val. There was no difference between the values of AID of all AA observed with either Cr_2O_3 or TiO_2.

6.5 Discussion

The appropriate choice of feed ingredients plays an important role in the strategic use of xylanase in non-ruminant animals (Adeola and Cowieson, 2011). Benefical effects of inclusion of exogenous xylanase have been reported in wheat-based diets in both poultry (Adeola and Bedford, 2004; Olukosi et al., 2007) and pigs (Barrera et al., 2004; Vahjen et al., 2007) due to the high concentration of non-starch polysaccharide (NSP) in wheat. Xylanase has also been evaluated in CSBM diets in both chickens and pigs (Stefanello et al., 2016; Cho et al., 2017). Similar to the results of several previous studies conducted in broiler chickens (Nian et al., 2011), no effect of exogenous xylanase on growth performance in broiler chickens was observed in the current study. However, Kiarie et al. (2014) reported that the addition of xylanase (1,250 xylanase unit/kg of diet) to the CSBM diet improved growth performance of broiler chickens, where more NSP substrate was provided by including corn distillers' dried grains with solubles. Therefore, the lower concentration of NSP might be the reason for the lower magnitude of response to supplemental enzyme in CSBM diets in the current study as discussed by Bedford and Schulze (1998).

Efficacy of supplemental xylanase on energy and nutrient digestion was influenced by the choice of FME in this study. In the broiler chicken study, supplemental xylanase in the diet containing sand did not show any beneficial effect, and the reason is unclear. Presumably, the grit effect of sand with large particle size was beneficial in the gizzard, which independently increased the ileal energy digestibility of diets (Liu and Fowler, 2017) by increasing the contact area between endogenous digestion enzyme and ileal digesta. In the current study, the chickens fed the diet containing sand had the greatest AID of energy and Lys regardless of the inclusion of xylanase, which also supports this speculation. Therefore, the inclusion of sand in the diet might restrain the potential improvement space for exogenous xylanase.

In the experimental diets containing DAE, inclusion of exogenous xylanase negatively affected the AID of His and Thr in broiler chickens and AID of Arg and Lys in pigs, and the reason is unclear. Exogenous xylanase could specifically cleave and hydrolyze the xylose backbone of arabinoxylans and release oligosaccharides consisting of xylose or xylose-arabinose residues (Svihus, 2010). On the other hand, due to the specific physical properties of DAE including high oil absorption capacity, large active surface, and high amorphous silicon dioxide content (Korunić, 1997), DAE has been included in poultry diets to treat parasites (Bennett et al., 2011). Furthermore, most of the indispensable AA which responded negatively to xylanase inclusion in the current study are basic AA (Nagai and Taniguchi, 2014) and share the y^+L transporter system (Bröer, 2008). Therefore, it is reasonable to speculate that the xylo-oligosaccharides released during the degradation of NSP by exogenous xylanase might function together with DAE as some unidentified factor associated with disturbing the process of either AA digestion or absorption.

The main substrate for exogenous xylanase is arabinoxylans (Svihus, 2010), which is the major component of NSP in wheat (Steenfeldt et al., 1998). The amount of soluble and insoluble

NSP in WB have been reported to be 14 and 350 g/kg (Yin et al., 2000a), and therefore the inclusion of WB increased the concentration of NSP in the diet, which might account for the beneficial effect of exogenous xylanase observed in both broiler chicken and pig experiments. Yin et al. (2000b) has reported that if a large amount of substrate is available to exogenous enzyme, there might be more scope for this enzyme to increase the nutrient digestibility. One of the possible modes of action of xylanase in wheat-based diets fed to poultry is decreasing the soluble NSP-induced viscosity of ileal digesta (Bedford and Schulze, 1998; Adeola and Bedford, 2004), which might also be the mode of action in the diet containing WB due to the soluble NSP in WB. Furthermore, another possible mode of action of xylanase in the diet containing WB might be the increased release of xylo-oligosaccharides from the degradation of NSP in WB contained in the diet, could have been utilized by beneficial bacteria, and as a result improved the utilization of nutrient (Liao and Nyachoti, 2017).

High dietary fiber level could increase the endogenous N losses by increasing the endogenous losses of epithelial cells or mucus secretion, which might be relieved by including exogenous enzymes (Sauer, 1976; Adeola, 2001; Gabert et al., 2001). Therefore, the modest improvement in the AID of Met and Pro in chickens fed the diet containing WB might be due to a reduction in the endogenous losses of these AA with the addition of xylanase as discussed by Adeola and Cowieson (2011). However, the beneficial effect of hydrolyzing fiber by exogenous xylanase was not evident for Lys, Arg, Ile, Leu, Val, and Phe in broiler chickens.

In the current pig experiment, a beneficial effect of exogenous xylanase in the diet containing WB was observed in AID of energy. In broiler chickens, it has been reported that the fermentable xylo-oligosaccharides from the hydrolysis of NSP by exogenous xylanase could be used by beneficial bacteria as prebiotics to increase the count of beneficial bacteria and the amount

of volatile fatty acids, which could contribute energy to host animals (Choct et al., 1996; Nian et al., 2011). Yin et al. (2000b) indicated that the effect from microbial xylanase on NSP degradation may be biologically significant, and the relatively long small intestine in pigs compared with chickens might increase the chance for microbial colonization in the small intestine. Therefore, it is reasonable to speculate that the prebiotics effect from hydrolysis by exogenous xylanase could be utilized by beneficial bacteria in the small intestine of pigs, as a result, the ileal digestibility of energy was increased in pigs. Furthermore, the main contributor to ileal endogenous loss of Lys in the pig originates from bacteria (Lien et al., 1997), and the increased population of beneficial bacteria might be the reason for the decrease in AID of Lys in pigs.

In broiler chickens, Olukosi et al. (2012) reported that the AID of AA in CSBM diets calculated with TiO_2 was greater than with Cr_2O_3 as DIM, whereas the AID of GE and AA observed with Cr_2O_3 was greater compared with TiO_2, but the ATTD of GE and DM observed with Cr_2O_3 and TiO_2 were similar. The varying results comparing Cr_2O_3 and TiO_2 might be related to the inclusion of different exogenous enzymes, which was phytase in the study reported by Olukosi et al. (2012) and xylanase in the current study. In pigs, greater AID and ATTD of energy in pigs fed CSBM diets was observed with TiO_2 compared with Cr_2O_3 as DIM (Wang and Adeola, 2018), however, in the current study no differences were noted. This is explained by a reduced interference from dietary fiber due to the hydrolysis of arabinoxylans backbone and reduced degree of polymerization with the addition of xylanase in this study (Adeola and Cowieson, 2011). Overall, the choice of DIM affected AID but not ATTU in broiler chickens; and had no effect on digestibility in pigs. This different effect of DIM type might be explained by dissimilar procedures of sampling, where ileal digesta samples of broiler chickens were collected one time following

euthanasia, whereas ileal digesta samples in pigs were collected over a 2-d period, which may be more representative of attainment of equilibrium.

6.6 Conclusion

In conclusion, the choice of formulation method for energy in experimental diets affects the evaluation of xylanase on efficiency of energy and nutrient in broiler chickens and growing pigs, and choice of digestibility index marker affects observed ileal digestibility values in broiler chickens.

6.7 Reference

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Table 6-1. Ingredient and analyzed nutrient composition of experimental diets for broiler chickens and pigs (g/kg as-fed basis)[1]

	Broiler chickens							Pigs						
Diet	PC	Sand		DAE		Wheat bran		PC	Sand		DAE		Wheat bran	
Xylanase		-	+	-	+	-	+		-	+	-	+	-	+
Ingredients														
Corn	569.2	554.1	524.1	554.1	524.1	538.4	508.4	560.2	545.8	515.8	545.8	515.8	539.6	509.6
Soybean meal	336.0	327.1	327.1	327.1	327.1	317.8	317.8	336.0	327.4	327.4	327.4	327.4	323.6	323.6
Soybean oil	36.0	10.0	10.0	10.0	10.0	10.0	10.0	45.0	18.0	18.0	18.0	18.0	3.0	3.0
Sand	-	50.0	50.0	-	-	-	-	-	50.0	50.0	-	-	-	-
DAE	-	-	-	50.0	50.0	-	-	-	-	-	50.0	50.0	-	-
Wheat bran	-	-	-	-	-	75.0	75.0	-	-	-	-	-	75.0	75.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
DL-Methionine	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
L-Lysine•HCl	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
L-Threonine	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Limestone	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
Monocalcium phosphate	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
Mineral-vitamin premix[2]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	-	-	-	-	-	-	-
Vitamin premix[3]	-	-	-	-	-	-	-	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[4]	-	-	-	-	-	-	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Selenium premix[5]	-	-	-	-	-	-	-	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide[6]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Titanium dioxide[6]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Xylanase premix[7]	-	-	30.0	-	30.0	-	30.0	-	-	30.0	-	30.0	-	30.0
Analyzed nutrient and energy														
Dry matter, g/kg	890.7	894.8	892.5	891.4	891.0	890.2	889.2	891.1	892.7	890.1	890.2	892.0	885.3	887.4
Crude protein, g/kg[8]	214.9	210.1	210.3	199.2	205.6	208.0	203.4	216.3	217.0	212.9	210.6	220.3	227.1	220.5
Gross energy, MJ/kg	16.3	14.8	15.0	14.8	15.0	15.8	15.9	16.7	15.1	15.1	14.9	14.8	15.6	15.6
Acid detergent fiber, g/kg	22.6	77.2	68.2	42.5	45.1	33.4	34.9	24.9	76.9	77.8	43.6	44.7	38.8	35.4
Neutral detergent fiber, g/kg	72.8	123.9	116.4	95.5	97.4	114.2	106.4	85.2	122.7	120.5	85.1	89.2	129.6	133.8
Indispensable amino acids														
Arg	14.0	13.6	14.3	13.5	13.8	14.2	14.0	14.0	13.7	13.6	14.0	14.1	14.4	14.6
His	5.4	5.3	5.5	5.9	5.2	5.5	5.5	5.5	5.4	5.2	5.3	5.5	5.7	5.7
Ile	9.2	9.3	9.8	9.9	9.3	9.4	9.2	9.5	9.4	9.0	9.1	9.6	9.7	9.8

Leu	17.7	17.1	17.7	17.9	17.2	17.5	17.3	17.7	17.4	17.0	17.3	17.5	17.7	18.0
Lys	14.4	14.1	14.8	14.5	13.9	14.3	14.3	14.6	14.5	14.1	14.2	14.5	14.5	14.8
Met	6.5	6.3	6.4	6.8	6.1	6.2	6.5	6.3	7.1	6.9	6.4	6.6	6.3	6.7
Phe	10.5	10.2	10.7	11.0	10.3	10.5	10.3	10.7	10.6	10.3	10.5	10.7	10.9	11.0
Thr	9.1	8.5	8.7	10.0	8.5	8.7	8.7	9.1	9.1	8.8	8.9	8.9	8.9	9.0
Trp	2.7	2.8	2.6	2.4	2.4	2.4	2.4	2.3	2.3	2.3	2.3	2.3	2.5	2.4
Val	9.9	10.1	10.4	10.8	10.0	10.2	10.1	10.1	10.0	9.7	9.8	10.4	10.6	10.7
Dispensable amino acids														
Ala	10.3	9.9	10.3	10.9	10.0	10.3	10.3	10.2	10.1	9.9	10.1	10.1	10.4	10.5
Asp	21.9	20.9	22.0	22.2	21.2	21.3	21.1	21.8	21.8	21.5	21.5	21.7	21.9	22.2
Cys	3.1	3.0	3.2	3.3	3.2	3.4	3.4	3.3	3.3	3.3	3.2	3.2	3.5	3.5
Glu	38.2	36.9	38.6	37.1	37.4	38.6	38.1	37.9	37.6	37.0	37.7	37.7	39.0	39.2
Gly	8.8	8.5	8.9	10.6	8.6	9.1	9.0	8.7	8.7	8.5	8.6	8.7	9.2	9.3
Pro	12.2	11.7	12.1	12.2	12.3	12.5	13.3	11.8	11.5	11.5	11.8	11.9	12.3	12.4
Ser	10.1	9.0	9.5	13.8	9.5	9.8	9.7	9.9	9.9	9.8	10.4	9.5	9.6	9.7
Tyr	7.4	7.1	7.4	8.0	7.2	7.2	7.1	7.5	6.8	7.3	7.5	7.4	7.3	7.5

[1]PC = positive control; DAE = diatomaceous earth.

[2]Provided the following quantities per kg of complete diet: vitamin A, 8,575 IU; vitamin D_3, 4,300 IU; vitamin E, 28.6 IU; menadione, 7.30 mg; riboflavin, 9.15 mg; $_D$-pantothenic acid, 18.3 mg; niacin, 73.5 mg; choline chloride, 1,285 mg; vitamin B_{12}, 0.02 mg; biotin, 0.09 mg; thiamine mononitrate, 3.67 mg; folic acid, 1.65 mg; pyridoxine hydrochloride, 5.50 mg; I, 1.85 mg; Mn, 180 mg; Cu, 7.40 mg; Fe, 73.5 mg; Zn, 180 mg; Se, 0.43 mg.

[3]Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D_3, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; $_D$-pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B_{12}, 0.02 mg.

[4]Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

[5]Provided 0.3 mg Se/kg of complete diet.

[6]Provided 5 g chromic oxide/kg and 5 g titanium dioxide/kg of complete diet.

[7]A 0.165 g xylanase (160,000 unit/g) was added to 29.835 g ground corn to form xylanase premix.

[8]CP = nitrogen × 6.25.

Table 6-2. The effect of xylanase (0 or 26,400 unit/kg of diet) and diet formulation method for energy (FME) on growth performance of broiler chickens[1]

			d 0 to 7			d 7 to 14			d 14 to 21			d 0 to 21		
FME	Xylanase	*n*	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg
Positive control		8	114.7	133.9	856.1	239.9	305.8	785.0	386.1	501.7	770.5	740.7	941.4	787.5
Sand	-	8	111.0	140.8	788.6	234.2	319.4	721.0	390.3	547.9	712.6	735.5	1008.0	729.3
Sand	+	8	103.3	133.3	776.7	226.9	311.3	727.4	398.1	550.4	723.3	728.4	994.9	731.9
Diatomaceous earth	-	8	99.9	132.0	756.5	214.0	295.8	720.6	380.9	530.0	721.0	694.8	957.8	725.8
Diatomaceous earth	+	8	98.3	130.8	751.8	217.4	294.7	735.9	338.7	487.5	690.1	654.4	913.0	715.9
Wheat bran	-	8	105.1	134.1	783.0	234.1	316.6	738.5	383.2	548.1	699.1	722.3	998.7	723.5
Wheat bran	+	8	108.5	135.1	803.2	241.4	322.8	747.2	400.0	570.8	700.3	749.8	1028.8	729.0
	-	24	105.3	135.6	776.0	227.4	310.6	726.7	384.8	542	710.9	717.5	988.2	726.2
	+	24	103.4	133.1	777.2	228.6	309.6	736.8	378.9	536.2	704.6	710.9	978.9	725.6
Sand		16	107.2^{A}	137.0	782.7^{AB}	230.6	315.3	724.2	394.2^{A}	549.1^{AB}	718.0	731.9^{A}	1001.5^{AB}	730.6
Diatomaceous earth		16	99.1^{B}	131.4	754.2^{B}	215.7	295.2	728.3	359.8^{B}	508.7^{B}	705.5	674.6^{B}	935.4^{B}	720.9
Wheat bran		16	106.8^{A}	134.6	793.1^{A}	237.7	319.7	742.8	391.6^{AB}	559.4^{A}	699.7	736.1^{A}	1013.7^{A}	726.2
SEM														
Xylanase			1.76	1.79	7.63	7.19	7.77	7.87	8.95	12.09	5.90	13.19	17.42	3.41
FME			2.01	2.04	9.32	8.81	9.52	9.63	10.84	14.57	7.23	16.15	21.34	4.17
P-value														
Positive control vs. others			< 0.001	0.865	< 0.001	0.380	0.769	< 0.001	0.795	0.090	< 0.001	0.289	0.203	< 0.001
Xylanase			0.335	0.195	0.909	0.914	0.929	0.369	0.634	0.727	0.453	0.722	0.709	0.900
FME			0.002	0.071	0.014	0.209	0.166	0.364	0.047	0.036	0.202	0.017	0.028	0.264
Xylanase × FME			0.081	0.188	0.445	0.834	0.868	0.945	0.117	0.257	0.116	0.341	0.467	0.388

[A,B]Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

[1]n = number of observations; G:F = gain to feed ratio.

Table 6-3. The effect of xylanase (0 or 26,400 unit/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) and apparent total tract utilization (ATTU) of nutrient and energy in broiler chickens[1]

				AID			ATTU	
FME	Xylanase	DIM	*n*	DM	CP	GE	DM	GE
Positive control			16	73.4	86.8	76.2	70.3	77.5
Sand	-		16	69.7	88.5^{X}	76.6	67.1	76.4
Sand	+		16	69.3	87.2^{Y}	76.6	70.8	75.6
Diatomaceous earth	-		16	69.5	86.2	75.4	69.3	75.9
Diatomaceous earth	+		16	68.9	86.8	75.1	69.7	74.0
Wheat bran	-		16	69.8	85.3	72.0	71.4	77.7
Wheat bran	+		16	70.5	85.2	72.9	71.4	78.0
	-		48	69.7	86.7	74.6	69.3	76.7
	+		48	69.6	86.4	74.9	70.7	75.9
Sand			32	69.5^{AB}	87.9	76.6^{A}	68.9^{B}	76.0^{B}
Diatomaceous earth			32	69.2^{B}	86.5	75.2^{B}	69.5^{AB}	74.9^{B}
Wheat bran			32	70.2^{A}	85.3	72.4^{C}	71.4^{A}	77.9^{A}
		Cr_2O_3	48	70.6	87.0	75.5	70.4	76.6
		TiO_2	48	68.7	86.1	74.0	69.5	75.9
SEM								
Xylanase				0.26	-	0.26	0.58	0.39
FME				0.30	-	0.31	0.71	0.47
Xylanase × FME				-	0.49	-	-	-
DIM				0.26	0.41	0.26	0.58	0.39
P-value[2]								
Positive control vs. others				< 0.001	0.673	0.012	0.818	0.226
Xylanase				0.787	0.362	0.487	0.097	0.150
FME				0.048	< 0.001	< 0.001	0.047	< 0.001
Xylanase × FME				0.175	0.031	0.297	0.129	0.308
DIM				< 0.001	0.032	< 0.001	0.262	0.207

^{A,B,C}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

^{X,Y}Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

[1]n = number of observations; DM = dry matter; CP = crude protein; GE= gross energy; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]The *P*-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

Table 6-4. The effect of xylanase (0 or 26,400 unit/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) of amino acids (AA) in broiler chickens[1]

				AID of indispensable AA										AID of dispensable AA							
FME	Xyl	DIM	*n*	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
PC			16	91.8	89.4	86.6	87.7	90.1	95.7	87.7	83.5	90.1	84.4	86.9	84.8	79.7	90.5	83.4	87.6	85.6	88.2
Sand	-		16	92.1	90.4	87.8	88.2	91.0	96.3	88.3	84.3	90.6^{X}	86.0	87.7	85.4	81.6	91.0	84.4	88.3	85.5	88.4
Sand	+		16	92.1	90.1	87.7	88.0	90.9	96.2	88.2	83.5	88.8^{Y}	85.8	87.5	85.2	81.0	91.0	84.0	87.8	85.5	88.2
DAE	-		16	90.9	89.1^{X}	87.9	88.0	90.3	96.1	88.6	85.3^{X}	88.2	85.4	87.9^{X}	85.5	80.1^{X}	90.3	86.0^{X}	86.3	89.6^{X}	89.1^{X}
DAE	+		16	91.4	87.5^{Y}	87.0	87.6	89.9	95.8	88.0	82.4^{Y}	87.7	84.4	86.9^{Y}	84.8	78.7^{Y}	90.6	82.6^{Y}	86.5	84.9^{Y}	87.9^{Y}
Wheat bran	-		16	90.8	87.8	85.8	86.4	88.8	94.9^{Y}	86.4	81.8	87.4	83.2	85.0	83.5	79.7	89.5	81.3	86.8^{Y}	84.1	86.7
Wheat bran	+		16	90.5	88.7	85.9	86.7	89.5	95.5^{X}	86.5	82.2	87.6	83.3	85.9	83.7	80.8	89.9	82.1	88.1^{X}	84.3	87.0
	-		48	91.2	89.1	87.2	87.5	90.0	95.8	87.8	83.8	88.7	84.9	86.9	84.8	80.5	90.3	83.9	87.2	86.4	88.1
	+		48	91.3	88.7	86.9	87.4	90.1	95.8	87.5	82.7	88.0	84.5	86.8	84.6	80.2	90.5	82.9	87.4	84.9	87.7
Sand			32	92.1^{A}	90.3	87.8^{A}	88.1^{A}	91.0^{A}	96.2	88.3^{A}	83.9	89.7	85.9^{A}	87.6	85.3^{A}	81.3	91.0^{A}	84.2	88.0	85.5	88.3
DAE			32	91.2^{AB}	88.3	87.4^{A}	87.8^{A}	90.1^{B}	96.0	88.3^{A}	83.9	87.9	84.9^{A}	87.4	85.2^{A}	79.4	90.5^{A}	84.3	86.4	87.3	88.5
Wheat bran			32	90.6^{B}	88.2	85.9^{B}	86.5^{B}	89.1^{C}	95.2	86.5^{B}	82.0	87.5	83.3^{B}	85.5	83.6^{B}	80.3	89.7^{B}	81.7	87.5	84.2	86.8
		Cr_2O_3	48	91.6	89.3	87.4	87.9	90.4	95.9	88.0	83.8	88.7	85.2	87.2	85.2	80.9	90.7	83.9	87.7	86.1	88.3
		TiO_2	48	91.0	88.6	86.6	87.1	89.8	95.7	87.3	82.7	88.0	84.2	86.4	84.2	79.7	90.1	82.9	86.9	85.2	87.5
SEM																					
Xyl				0.28	-	0.25	0.24	0.22	-	0.23	-	-	0.53	-	0.23	-	0.19	-	-	-	-
FME				0.32	-	0.28	0.26	0.24	-	0.26	-	-	0.56	-	0.26	-	0.21	-	-	-	-
Xyl × FME				-	0.33	-	-	-	0.20	-	0.42	0.33	-	0.39	-	0.49	-	0.40	0.37	0.39	0.29
DIM				0.28	0.23	0.25	0.24	0.22	0.14	0.23	0.29	0.22	0.53	0.27	0.23	0.34	0.19	0.29	0.27	0.28	0.21
P-value[2]																					
PC vs. others				0.354	0.258	0.312	0.630	0.823	0.735	0.949	0.580	0.001	0.569	0.826	0.844	0.278	0.783	0.968	0.554	0.919	0.315
Xyl				0.758	0.142	0.308	0.632	0.708	0.775	0.360	0.002	0.007	0.282	0.750	0.388	0.363	0.378	0.003	0.259	< 0.001	0.076
FME				0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Xyl × FME				0.600	0.001	0.273	0.493	0.094	0.048	0.516	< 0.001	0.005	0.298	0.029	0.268	0.020	0.646	< 0.001	0.020	< 0.001	0.020
DIM				0.113	0.011	0.008	0.006	0.008	0.092	0.008	0.004	0.009	0.011	0.010	0.002	0.004	0.006	0.003	0.009	0.006	0.002

[A,B,C]Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

[X,Y]Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

[1]n = number of observations; PC = positive control; DAE = diatomaceous earth; Xyl = xylanase; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]The *P*-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

Table 6-5. The effect of xylanase (0 or 26,400 unit/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrient and energy in pigs[1]

				AID			ATTD	
FME	Xylanase	DIM	*n*	DM	CP	GE	DM	GE
Positive control			12	68.9	76.7	71.6	86.8	88.4
Sand	-		12	68.5^{X}	78.1	71.6	82.0	88.0
Sand	+		12	66.0^{Y}	77.4	70.0	81.4	88.0
Diatomaceous earth	-		12	65.9	78.4	70.7	81.2	87.6
Diatomaceous earth	+		12	65.3	79.1	71.3	82.0	87.7
Wheat bran	-		12	65.1	76.3	66.3^{Y}	83.0	84.6
Wheat bran	+		12	66.1	76.4	68.5^{X}	83.2	84.4
	-		36	66.5	77.6	69.5	82.1	86.7
	+		36	65.8	77.6	69.9	82.2	86.7
Sand			24	67.3	77.8	70.8	81.7^{B}	88.0^{A}
Diatomaceous earth			24	65.6	78.8	71.0	81.6^{B}	87.7^{A}
Wheat bran			24	65.6	76.3	67.4	83.1^{A}	84.5^{B}
		Cr_2O_3	36	66.3	77.7	69.9	82.2	86.8
		TiO_2	36	66.0	77.5	69.6	82.0	86.6
SEM								
Xylanase				-	0.57	-	0.48	0.67
FME				-	0.78	-	0.51	0.70
Xylanase × FME				0.73	-	0.81	-	-
DIM				0.56	0.65	0.65	0.48	0.67
P-value[2]								
Positive control vs. others				0.110	0.554	0.229	0.009	0.090
Xylanase				0.046	0.916	0.199	0.573	0.925
FME				0.110	0.061	0.001	0.001	< 0.001
Xylanase × FME				0.005	0.142	0.004	0.112	0.845
DIM				0.693	0.786	0.706	0.492	0.590

^{A,B}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

^{X,Y}Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

[1]n = number of observations; DM = dry matter; CP = crude protein; GE= gross energy; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]The *P*-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

Table 6-6. The effect of xylanase (0 or 26,400 unit/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) of amino acids (AA) in pigs[1]

				AID of indispensable AA									AID of dispensable AA							
FME	Xyl	DIM	*n*	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
PC			12	90.4	85.2	82.4	82.8	86.6	92.1	83.3	76.8	80.2	76.1	77.0	79.9	72.1	85.4	70.6	80.9	81.6	83.4
Sand	-		12	89.1^{Y}	86.2	83.5	83.6	86.4	94.0	83.6	78.5	80.5	77.4	78.7	81.3	72.5^{Y}	86.0	74.0	82.6	82.8	82.6^{Y}
Sand	+		12	90.5^{X}	86.7	82.6	84.1	87.0	92.9	84.2	78.7	81.4	78.0	78.5	80.8	74.7^{X}	85.3	72.8	82.5	83.7	84.9^{X}
DAE	-		12	91.4^{M}	86.7	83.3	84.1	88.8^{X}	93.3	84.1	78.9	81.7	77.2	79.4	81.4	73.0	87.3^{X}	74.1	81.4	83.9^{X}	85.0^{M}
DAE	+		12	89.9^{N}	86.3	83.3	83.9	87.2^{Y}	92.2	84.2	78.6	81.3	78.7	78.3	81.0	72.5	85.3^{Y}	73.6	82.3	82.4^{Y}	83.8^{N}
Wheat bran	-		12	89.2	86.0	83.0	82.9	86.5^{M}	92.9	82.5	76.9	80.2	76.6	78.2	79.6	73.0	85.2	72.6	82.0	81.5^{M}	82.7
Wheat bran	+		12	88.6	85.5	82.3	82.5	85.4^{N}	92.3	82.8	76.3	79.7	77.1	76.4	78.6	72.4	84.2	72.4	81.6	79.5^{N}	82.7
	-		36	89.9	86.3	83.3	83.5	87.2	93.4	83.4	78.1	80.8	77.1	78.8	80.8	72.8	86.1	73.6	82.0	82.7	83.4
	+		36	89.7	86.2	82.7	83.5	86.5	92.5	83.7	77.9	80.8	77.9	77.7	80.1	73.2	85.0	72.9	82.1	81.9	83.8
Sand			24	89.8	86.5	83.1	83.9	86.7	93.4	83.9	78.6	81.0	77.7	78.6	81.0^{AB}	73.6	85.7	73.4	82.5	83.3	83.8
DAE			24	90.6	86.5	83.3	84.0	88.0	92.8	84.2	78.8	81.5	78.0	78.8	81.2^{A}	72.7	86.3	73.9	81.8	83.2	84.4
Wheat bran			24	88.9	85.8	82.7	82.7	85.9	92.6	82.7	76.6	79.9	76.8	77.3	79.1^{B}	72.7	84.7	72.5	81.8	80.5	82.7
	Cr_2O_3		36	89.8	86.3	83.1	83.6	86.9	93.0	83.7	78.1	80.9	77.6	78.3	80.5	73.1	85.6	73.4	82.1	82.4	83.7
	TiO_2		36	89.7	86.2	82.9	83.4	86.8	92.9	83.5	77.9	80.7	77.4	78.2	80.4	72.9	85.5	73.1	82.0	82.2	83.6
SEM																					
Xyl				-	0.55	0.51	0.48	-	0.30	0.44	0.55	0.69	0.54	0.70	0.51	-	-	1.25	0.94	-	-
FME				-	0.70	0.69	0.66	-	0.39	0.61	0.79	0.88	0.80	0.99	0.66	-	-	1.42	1.08	-	-
Xyl × FME				0.47	-	-	-	0.91	-	-	-	-	-	-	-	1.32	0.89	-	-	0.75	0.66
DIM				0.39	0.61	0.58	0.55	0.81	0.34	0.51	0.65	0.76	0.65	0.82	0.56	1.09	0.82	1.31	1.27	0.60	0.53
P-value[2]																					
PC vs. others				0.413	0.411	0.622	0.558	0.822	0.299	0.799	0.483	0.704	0.433	0.535	0.617	0.685	0.848	0.232	0.568	0.590	0.877
Xyl				0.063	0.572	0.022	0.744	0.003	< 0.001	0.110	0.427	0.984	0.014	0.004	0.030	0.340	< 0.001	0.093	0.609	0.007	0.077
FME				0.017	0.556	0.720	0.237	0.090	0.163	0.124	0.087	0.263	0.539	0.381	0.025	0.760	0.044	0.503	0.691	0.009	0.118
Xyl × FME				< 0.001	0.084	0.281	0.239	0.001	0.141	0.496	0.607	0.099	0.276	0.092	0.678	0.016	0.017	0.595	0.172	0.004	< 0.001
DIM				0.815	0.850	0.839	0.830	0.864	0.879	0.820	0.821	0.834	0.845	0.858	0.768	0.842	0.732	0.800	0.942	0.822	0.826

[A,B]Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

[X,Y] Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

[M,N] Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

[1]n = number of observations; PC = positive control; DAE = diatomaceous earth; Xyl = xylanase; Cr_2O_3 = chromic oxide; TiO_2 = titanium dioxide.

[2]The *P*-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

CHAPTER 7. SUMMARY

7.1 Summary

Accurate evaluation of the nutritional value of feed ingredients plays a critical role to meet the requirement of animals as well as avoid feed wastage. Apparent ileal digestibility (AID), standard ileal digestibility (SID), and apparent total tract digestibility (ATTD) are important indicators to evaluate the digestibility of energy and nutrient. Index method has been widely used to determine AID, SID, and ATTD in pigs and broiler chickens because the index method is less laborious compared with the total collection method. Index method requires the inclusion of digestibility index marker (DIM), and therefore it is important to investigate the interaction between DIM type and dietary characteristics on the determination of energy and nutrient utilization in pigs and chickens. In this dissertation, we investigated the effect of type and level of DIM and dietary characteristics including dietary fiber type, dietary protein sources, and inclusion of xylanase on energy and nutrient utilization in pigs and broiler chickens.

In chapter 1, the methodology of the determination of ileal and total tract utilization of amino acids (AA) and energy in pigs and broiler chickens is reviewed. Inclusion of DIM in the diet is necessary to determine the ileal and total tract utilization with the index method. Acid insoluble ash (AIA), chromic oxide (Cr_2O_3), and titanium dioxide (TiO_2) are the three commonly used DIM in non-ruminant animals. The ideal DIM should meet several characteristics including non-absorbable, completely inert, regularly and completely voided in the feces, and uniformly mixed with the feed and feces (Adeola, 2001). Based on this assumption, the recovery of DIM should be 100%, however, not all the DIM could be recovered in outputs of animals (Jagger et al., 1992; Kavangh et al., 2001). The possible reasons for the low recovery of DIM might relate to

DIM type, chemical measurement method for DIM, and dietary characteristics. As a result, the determination of ileal or total tract digestibility might also be influenced by the choice of DIM and various dietary characteristics including fiber type, protein ingredients, and inclusion of exogenous enzyme. To investigate the effect of DIM type, multiple DIM were included in each diet to eliminate the effect of experimental animals on results.

Chapter 2 included one study conducted in growing pigs to investigate the effect of combination of DIM and fiber on gross energy (GE) and nitrogen (N) digestibility and recovery of DIM. Three corn-soybean meal-based diets were formulated with corn starch, corn bran or oat bran at 100 g/kg. Acid insoluble ash, Cr_2O_3, and TiO_2 were included as DIM in each diet. The AID and ATTD of N and GE and the recovery of DIM were determined. There were interactions between DIM type and dietary fiber type for all the responses. The choice of DIM affected the determination of AID of N and GE within each diet, but the ATTD of N or GE within each diet was less affected by DIM type. Furthermore, the recovery of TiO_2 in feces of pigs fed the oat bran was 78.3%, which was the least among all the diets.

In chapter 3, a study was conducted to investigate the effect of dietary fiber, ileal digesta collection day, and time period on the concentration pattern of DIM. The samples used in this study were collected in the previous study in chapter 2. The concentrations of AIA, Cr, and Ti were analyzed in each ileal digesta sample. The ileal digesta collection day had limited effect on DIM concentration and the three DIM moved synchronously in diets irrespective of time period.

In chapter 4, a study was conducted to investigate if the ileal and total tract digestibility of N and GE and recovery of DIM were influenced by inclusion level and type of DIM, and inclusion level of OB. Six diets were formulated as a 2 × 3 factorial arrangement with two levels of OB (0 or 100 g/kg) and three levels of DIM (2.5, 5.0, or 7.5 g/kg). The AID of GE and N, the ATTD of

GE, and the recovery of Cr_2O_3 or TiO_2 were affected by DIM type, but not DIM level; the inclusion of OB had no effect on AID of GE and N, and DIM recovery; and the duration of fecal collection had no effect on ATTD of GE, and DIM recovery.

Chapter 5 included a study conducted in growing pigs to investigate the additivity of AID and SID of crude protein (CP) and AA in mixed diets containing wheat, and multiple protein sources fed to pigs. The basal ileal endogenous losses (BEL), AID, and SID of AA were determined by Cr_2O_3 and TiO_2. The measured and predicted AID and SID of AA in mixed diets [wheat-canola meal (CM)-meat and bone meal (MBM), wheat-MBM-sorghum distillers' dried grains with solubles (DDGS), and wheat-CM-MBM-DDGS] were compared. The results indicated that the determination of BEL, AID, and SID of CP and AA, as well as the additivity of AID and SID of CP and most indispensable AA in mixed diets were not affected by DIM type. In addition, more accurate prediction of ileal digestibility of AA was achieved using SID rather than AID in mixed diets containing wheat, CM, MBM, and DDGS.

In chapter 6, a study investigated the growth performance and nutrient utilization responses of broiler chickens and the nutrient utilization of pigs to xylanase, experimental diet formulation method for energy (FME), and DIM. In Exp. 1, a total of 448 male broiler chickens were used in a randomized complete block design with BW as a blocking factor. Seven dietary treatments were prepared in a 3 × 2 + 1 factorial arrangement with inclusion of sand, diatomaceous earth, or wheat bran as FME and without or with xylanase (26,400 unit/kg of diet) plus positive control. Each of Cr_2O_3 and TiO_2 were incorporated at 5 g/kg in diets. In Exp. 2, twenty-one barrows (initial BW = 33.0 ± 0.3 kg), fitted with simple T-cannulas at the distal ileum, were used in a triplicate 7 × 2 incomplete Latin Square design with 7 dietary treatments, which were prepared by the same arrangement as in broilers. The efficacy of xylanase on ileal energy and AA digestibility depends

on the choice of formulation method for energy in broilers and pigs, and DIM affects ileal digestibility in broilers.

An important assumption about DIM is that DIM should uniformly mix within the feed and are uniformly excreted in feces to ensure that the collected sample is representative (Adeola, 2001). It has been reported that the choice of DIM could affect the determination of AID and ATTD of nutrient in pigs (Jagger et al., 1992; Kavanagh et al., 2001; Favero et al., 2014), which violates the assumption of DIM. What's more, the recovery of DIM was affected by the type of dietary fiber (Köhler et al., 1990). Therefore, it is reasonable to speculate that there might be interaction between DIM type and dietary fiber type on the determination of AID and ATTD of GE and N. The results in chapter 2 indicated the DIM should be carefully chosen to determine AID, especially for the diet which is rich in soluble fiber. Furthermore, the concentration pattern of DIM in diets containing different fiber sources across time period on different ileal digesta collection days was similar to each other and any of the three DIM was an equivalent choice in the digestibility study.

Except DIM type, the inclusion level of DIM in diet is inconsistent and therefore it might also affect the determination of nutrient digestibility (Olukosi et al., 2012). The results in chapter 4 showed that including 2.5, 5.0, or 7.5 g/kg DIM in diet did not affect the determination of AID and ATTD of nutrient, but the choice of DIM affected the results. Furthermore, the study in chapter 2 indicated the addition of 100 g/kg oat bran decreased the recovery of TiO_2 from 88.2% to 78.5%, however, this negative effect of OB was not observed in the study in chapter 4.

From the previous studies in chapter 2 and 4, the choice of DIM could affect the determination of ileal digestibility, consequently the additivity of ileal digestibility of CP and AA in mixed diets. In addition, chromic oxide has been generally used as DIM in previous studies that

investigated the assumption of additivity of AID and SID of CP and AA in mixed diets, however, there is a scarcity of data with TiO_2 as DIM (Fan et al., 1993; Stein et al., 2005; Xue et al., 2014). The study in chapter 5 indicated that the choice of DIM has no effect on the additivity of AID and SID of CP and most indispensable AA in all three mixed diets.

The main function of xylanase is to hydrolyze dietary fiber (Adeola and Cowieson, 2011), which might interfere with the interaction between fiber and DIM, and it is reasonable to speculate that the effect of FME and xylanase may depend on the choice of DIM. The results in chapter 6 indicated that the choice of DIM affects the ileal digestibility values in broiler chickens, but not in pigs.

The choice of DIM affected the determination of ileal digestibility of nutrient in pigs from chapters 2 and 4 and in chickens from chapter 6, but it had no effect on the determination of ileal and total tract digestibility of nutrient in pigs from chapters 5 and 6 and total tract utilization in chickens from chapter 6. The reason that caused the inconsistent effect of DIM on the determination of nutrient utilization might relate to different format of the basal diet or different species of experimental animals, and further study is required. Furthermore, the dietary DIM could not be completely recovered in outputs and more studies should be conducted to investigate the reasons for this unsatisfactory result. At last, the total tract digestibility of nutrient determined by the index method is less than those determined by the total collection method, and the difference between these two methods might be decreased by using the empirically determined recovery as a correction factor.

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Research Interests

Interaction between digestibility index marker and dietary characteristics on the determination of energy and nutrient digestibility in pigs and broiler chickens;

Nutritional evaluation of carbohydrase enzymes in broiler chickens;

Development of prediction equations of energy values of feed ingredient in pigs.

PUBLICATIONS

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