FACTORS AFFECTING AMINO ACID DIGESTIBILITY IN MONOGASTRIC ANIMALS

by

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A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Animal Sciences

West Lafayette, Indiana

May 2020

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This dissertation is dedicated to my beloved parents, grandmother, and brother.

Special thanks to my fiancé Tingting.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to Dr. Layi Adeola who always advises and guides me to expand my ideas not only for research but also for my future career and life. His endless supports always made me move forward and encouraged me to work harder. I believe all the knowledge that I have learned under Dr. Adeola's mentoring is the most precious experience I have earned during my Ph. D. program. I truly appreciate the committee members of my Ph. D. program, Drs. Ajuwon, Radcliffe, and Ragland. Their kind support and valuable advice helped me to improve my knowledge and successfully conduct research.

I could have not conducted all the studies for the dissertation without the selfless supports of my colleagues in the Adeola Nutrition Lab. I would like to express my appreciation to Pat Jaynes who provides technical support and manages the lab with perfection. Thanks to colleagues whom I had an opportunity to work with including Nathan Horn, Pengcheng Xue, Fengrui Zhang, Yu Shi, Hang Lu, Tingting Wang, Saheed Osho, Olufemi Babatunde, Ayodeji Aderibigbe, Abidemi Adekoya, Jing Yuan, Sakkariya Nalukudy, Opeadura Osunbami, Chengkun Fang, Laura Beeson, Ruqing Zhong, Qifang Yu, and Vitor Haetinger, I really enjoyed working in the lab, and I will never forget all the good memories we made together. I also appreciate the members in the Animal Science Research and Education Center, Brian Ford, Jim Emilson, Aaron Nally, Katelin Ade, Jason Fields, Mike Zeltwanger, and Tyler Raber, and Mary Larimore in the Life Science Animal Building. They always put all their efforts to successfully conduct my research. In addition, I must thank Marlene Miller, Kim See, Pat Jonas, JoAnn Galyon, Shelly Gamble, and Kiersti Cunningham in the Department of Animals Sciences who supported my departmental business. I sincerely appreciate all the people whom I met and had wonderful times together with.

I would like to thank my parents, brother, and grandmother for supporting me in my study and work. Even though they are far away from me now, their love and support always motivated me to stay stronger. Special thanks to my fiancé, Tingting who always stood by me as a senior graduate student, mentor, and life companion. Without the support and trust of people whom I love, I could have not successfully completed this dissertation. I truly appreciate them, and this dissertation is dedicated to them.

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LIST OF ABBREVIATIONS

SYMBOL	DESCRIPTION
AA	Amino acid
ADF	Acid detergent fiber
ADG	Average daily gain
AEE	Acid-hydrolyzed ether extract
AID	Apparent ileal digestibility
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
$b^{0,+}AT$	Na+-independent cationic and zwitterionic amino acid transporter
BEL	Basal ileal endogenous losses
BW	Body weight
CE	Canola expellers
CM	Canola meal
CP	Crude protein
CV	Coefficient of variation
DDGS	Corn distillers' dried grains with solubles
DM	Dry matter
DMI	Dry matter intake
FDPP	Flash-dried poultry protein
FFCS	Full-fat canola seeds
FFSB	Full-fat soybean
GE	Gross energy
GIT	Gastrointestinal tract
HFM	Hydrolyzed feather meal
MBM	Meat and bone meal
mRNA	Messenger ribonucleic acid
mTORC1	Mechanistic target of rapamycin C1

N	Nitrogen
n	Number of observations
NDF	Neutral detergent fiber
NFD	Nitrogen-free diet
NRC	National Research Council
PBM	Poultry byproduct meal
PepT1	Intestinal peptide transporter 1
PM	Poultry meal
PNF	Peanut flour
PVTC	Post valve T-cecum cannula
RMSE	Root mean square error
SBM	Soybean meal
SBM-43	Soybean meal containing 430 g/kg crude protein
SBM-47	Soybean meal containing 470 g/kg crude protein
SD	Standard deviation
SE	Standard error
SED	Standard error of the differences of means
SEM	Standard error of the mean
SID	Standardized ileal digestibility
TEL	Total ileal endogenous losses
TIA	Trypsin inhibitor activity
TIU	Trypsin inhibitor unit

ABSTRACT

The objective of the experiments conducted for this dissertation was to determine the standardized ileal digestibility (SID) of amino acids (AA) in a variety of feed ingredients for broiler chickens and pigs. The effects of casein in experimental diets on the SID of AA in corn distillers' dried grains with solubles (DDGS) fed to pigs were evaluated. The SID of AA in feed ingredients, which include full-fat soybean (FFSB), two soybean meals (SBM), peanut flour (PNF), full-fat canola seeds (FFCS), canola meal (CM), canola expellers (CE), hydrolyzed feather meal (HFM), flash dried poultry protein (FDPP), poultry meal (PM), and meat and bone meal (MBM), were compared in broiler chickens and pigs. One of the studies determined the ileal digestibility of AA in casein by regression analysis and investigated the effects of 60 g/kg casein in experimental diets on the SID of AA in DDGS. The ileal digestibility of AA in casein were close to 100%, ranging from 95.5% (SE = 9.10) for Cys to 103.1% (SE = 4.40) for Arg. In addition, the SID of Lys and Phe in DDGS determined by pigs fed the diet containing DDGS and casein were greater ($P < 0.05$) than the values determined by pigs fed the diet containing DDGS without casein. Based on the results of this experiment, two additional experiments were conducted to determine the effects of graded concentrations of casein from 55 to 165 g/kg in experimental diets on the SID of AA in DDGS and to determine the effects of dietary DDGS concentrations (i.e., 155.6 or 466.8 g/kg) and addition of casein in experimental diets on the SID of AA in DDGS. The SID of indispensable AA, except for Arg and Lys, linearly decreased ($P < 0.05$) as the concentration of casein in experimental diets increased. Moreover, pigs fed the diets containing 155.6 g/kg DDGS had less ($P < 0.05$) SID of indispensable AA, except for Trp, in DDGS than those fed the diets containing 466.8 g/kg DDGS regardless of the addition of casein in experimental diets. Therefore, it may be concluded that the addition of casein improves the SID of AA in DDGS, but reduced DDGS concentration in experimental diets decreases the SID of AA in DDGS. In one pair of experiments conducted to compare the SID of AA in FFSB, SBM containing 430 g/kg crude protein, SBM containing 470 g/kg crude protein, and PNF between broiler chickens and pigs, the SID of AA, except for Trp, Ala, and Glu, in test ingredients for pigs were greater ($P < 0.05$) than the values for broiler chickens. In addition, in both broiler chickens and pigs, the SID of Ile, Leu, and Val in FFSB were less ($P < 0.05$) than in the other test ingredients. In another pair of experiments conducted to compare the SID of AA in FFCS, CM, and CE between broiler chickens and pigs, interactions ($P < 0.05$)

between experimental diets and species were observed in the SID of AA, except for Lys, Gly, Pro, and Ser. The SID of AA in FFCS for broiler chickens were greater ($P < 0.05$) than pigs; however, there was no difference in the SID of AA in CM or CE between broiler chickens and pigs. The objective of a third pair of experiments was to compare the SID of AA in HFM, FDPP, PM, and MBM fed to broiler chickens and pigs. There were interactions ($P < 0.05$) between experimental diets and species in the SID of His, Thr, Trp, and Val. In broiler chickens, the SID of His, Thr, and Trp in FDPP and PM were greater ($P < 0.05$) than in HFM but were less ($P < 0.05$) than MBM; however, difference in SID of His, Thr, and Trp among FDPP, PM, and MBM was not observed in pigs. Based on the results of three pairs of studies, it was revealed that differences in SID of AA in common feed ingredients for both broiler chickens and pigs were affected by species. Therefore, it may be concluded that the effects of feed ingredient-specific factors on the SID of AA are different between broiler chickens and pigs.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Broiler chickens and pigs are major non-ruminant animals in the livestock industry for meat production. To maximize meat production, growth of monogastric animals is of importance, which is mainly achieved through protein accretion [National Research Council (**NRC**), 2012]. Therefore, an adequate supply of proteins and amino acids (**AA**) from the diet is necessary to optimize the growth of monogastric animals and maintain normal physiological conditions. However, excessive supply of dietary proteins increases excretion of nitrogen (**N**), leading to environmental pollution. In addition, increased concentration of protein in diets for monogastric animals may increase feed cost, which ultimately reduces the profit of overall production. Therefore, a thorough understanding of AA utilization by monogastric animals is required to accurately supply proteins and AA in a cost-effective manner while minimizing impacts on the environment.

An accurate determination of available AA in feed ingredients is an essential prerequisite for the optimal supply of proteins and AA to broiler chickens and pigs. Due to the ease of methodology and additivity in mixed diets, the standardized ileal digestibility (**SID**) of AA has been widely used to describe the availability of AA in feed ingredients and diets for both broiler chickens and pigs (Kong and Adeola, 2014). However, most of the previous studies conducted to determine the SID of AA in feed ingredients have used semi-purified diets containing feed ingredients of interest as the sole source of N, which may cause deficiency of AA if feed ingredients of interest do not contain enough available AA to maintain the normal physiological condition of animals.

The SID of AA has been used for both broiler chickens and pigs due to the similarity of their digestive physiology. In addition, commercial diets for broiler chickens and pigs have been generally produced with common feed ingredients such as corn, wheat, or soybean meal (**SBM**). However, differences in digestive organs between broiler chickens and pigs may affect the SID of AA in common feed ingredients. Moreover, the extent to which feed ingredient-specific factors affect the SID of AA may be different between broiler chickens and pigs. Therefore, direct comparison of the SID of AA in common feed ingredients between broiler chickens and pigs is

required for understanding differences in utilization of AA between broiler chickens and pigs. In this chapter, general digestive physiology of AA and AA requirements of broiler chickens and pigs as well as methodology to determine the availability of AA in feed ingredients are reviewed to address the background of the dissertation and clarify the research gap in current monogastric nutrition.

1.2 Proteins and Amino Acids

Broiler chickens and pigs directly digest dietary proteins in the gastrointestinal tract (**GIT**) using endogenous enzymes. In general, the concentration of crude protein (**CP**) in commercial diets for broiler chickens and pigs ranges from 70 to 230 g/kg, depending on age, body weight (**BW**), and physiological states (NRC, 1994, 2012). The concentration of CP is calculated as the product of the concentration of N and 6.25 based on the assumption that the mean concentration of N in proteins is 160 g/kg and all N reside in proteins (Lewis, 2001). However, the concentration of N varies among proteins depending on the AA composition. The concentration of N in foods ranges from 157 g/kg for milk to 183 g/kg for peanuts (NRC, 2012). Moreover, N in feed ingredients may be as nonprotein N which results in overestimation of CP concentration (Lewis, 2001). Nevertheless, the concentration of CP has been used to describe the protein contents in feed ingredients and diets due to the representativeness and simplicity of analysis.

1.2.1 Classification of Amino Acids

There are 20 primary AA in dietary proteins which are required for biological functions in broiler chickens and pigs. Amino acids can be classified by the chemical structure of side chain, polarity, metabolism, and nutritional necessity (Table 1.1). Based on the chemical structure, AA can be divided into aliphatic, aromatic, and heterocyclic AA (Pond et al., 2005). Among aliphatic AA, Ile, Leu, and Val contain a branch in side chains, and therefore, these AA are referred to as branched-chain AA. In addition, two sulfur-containing AA, Met and Cys, contain S in side chains. Side chains of aromatic AA are composed of a phenyl group. On the other hand, heterocyclic AA are characterized by their side chains which contain a ring structure with C and N atoms. Together with the chemical structure of side chains, polarity of AA drives the transport of AA in the body of monogastric animals. Neutral AA contain either nonpolar or uncharged polar side chains;

however, charged polar side chains carry either acidic or basic side chains (Voet et al., 2008). Absorbed AA are used for protein synthesis and other metabolic reactions including transamination and deamination. In catabolic reactions of AA, glucogenic AA produce metabolites which can be involved in glucose metabolism, whereas ketogenic AA produce α-keto acids which can be converted to ketone body (D'Mello, 2003).

Among various criteria to classify AA, classification of AA based on nutritional necessity is the most prevalent in non-ruminant animal nutrition. Indispensable AA need to be provided by diets due to the insufficient or lack of de novo synthesis of carbon skeletons and α-keto acids, whereas dispensable AA can be sufficiently synthesized by metabolism of animals (D'Mello, 2003). Some AA including Arg, Cys, Pro, and Tyr have been considered as conditionally indispensable AA due to its insufficient synthesis during early age or conversion of Met and Phe to Cys and Tyr, respectively (Lewis, 2001).

1.2.2 Digestion of Proteins and Amino Acids

Ingested dietary proteins and AA enter the foregut where the first enzymatic digestion of dietary proteins occurs. One of the distinct differences in the GIT between broiler chickens and pigs is the digestive organs in the foregut. In broiler chickens, the digestive organs in the foregut consist of the esophagus, crop, proventriculus, and gizzard, whereas in pigs, those in the foregut consist of the esophagus and stomach.

The major function of the crop in broiler chickens is for temporary storage and lubrication of ingested diets. Depending on the presence of digesta in the gizzard, ingested diets are stored in the crop or bypass the crop and directly enter the proventriculus (Rodrigues and Choct, 2018). In the proventriculus, pepsinogen and HCl are secreted from oxynticopeptic cells, and the first enzymatic digestion of dietary proteins occurs (Denbow, 2000). The gizzard is structured with a dense muscular layer which enhances mechanical digestion to reduce the particle size of digesta (Rodrigues and Choct, 2018). Koilin, which is glycoprotein produced by mucosal glands, covers the interior surface of the gizzard to protect the membrane surface against HCl, proteolytic enzymes, and damage from mechanical digestion processes (Denbow, 2000).

In pigs, ingested diets directly flow into the stomach by peristaltic movement of the esophagus. The stomach of pigs can be divided into 4 regions: esophageal, cardiac gland, fundic gland, and pyloric regions (Yen, 2001). The esophageal region is considered as an extension of the

esophagus and does not have a mucous layer on the surface due to the lack of glandular cells. On the other hand, mucous-secreting cells are located in the cardiac region which protects against the acidic condition of digesta and proteolytic enzymes. In the membrane surface of the fundic gland region, parietal cells and chief cells produce HCl and pepsinogen, respectively, which hydrolyze the dietary proteins in digesta. The pyloric region also contains chief cells, but not parietal cells, and the majority of glandular cells in the pyloric region is mucous-secreting cells.

Low pH in the foregut induced by the secretion of HCl causes conformational changes in the complex structure of proteins, which enhances the enzymatic digestion. In both broiler chickens and pigs, pepsin is secreted as a zymogen, pepsinogen, which can be activated by low pH in the gut or autocatalytic reaction (Tang, 2013). Pepsin carries a broad substrate specificity and is classified as an endopeptidase which hydrolyzes the middle of the polypeptide chain (Tang, 2013). Crévieu-Gabriel et al. (1999) compared the activity of pepsin between broiler chickens and pigs using an in vitro protein solubility and reported that the pH sensitivity of pepsin derived from broiler chickens was greater than that of pepsin derived from pigs when hemoglobin, pea concentrate, and wheat gluten were used as substrates. However, the optimal pH of pepsin from broiler chickens was comparable with that from pigs (Crévieu-Gabriel et al., 1999).

The structure and function of the small intestine is similar between broiler chickens and pigs. Digesta from the stomach enters the duodenum and is mixed with pancreatic juice which contains a variety of zymogens of proteolytic enzymes including trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase A, and procarboxypeptidase B (Pond et al., 2005). Trypsinogen is activated to trypsin by enterokinase secreted from the brush border membrane of the duodenum (Denbow, 2000; Yen, 2001). All zymogens in the pancreatic juice are activated by trypsin including trypsinogen which can be activated by autocatalytic reaction. Among proteolytic enzymes in the pancreatic juice, trypsin, chymotrypsin, and elastase are categorized as endopeptidases, whereas carboxypeptidases A and B are categorized as exopeptidases which hydrolyze the carboxyl terminal of polypeptides. Trypsin has a strong affinity to peptide bonds consisting of basic AA (i.e., Arg and Lys) as carboxyl group (Baird and Craik, 2013). Chymotrypsin specifically hydrolyzes the peptide bond consisting of Leu, Phe, Trp, and Tyr as carboxyl group (Gráf et al., 2013). Elastase mainly cleaves the peptide bond consisting of Val, Ala, Gly, Ser, and Tyr as carboxyl group (de Oliveira and Salgado, 2013). On the other hand, carboxypeptidase A acts on the carboxyl terminal end of polypeptide chains containing aromatic

and branched-chain AA residues (Auld, 2013), whereas carboxypeptidase B specifically hydrolyzes the carboxyl terminal of Arg and Lys of polypeptide chain (Avilés and Vendrell, 2013). Polypeptides in digesta from the stomach are digested to shorter polypeptide chains or free AA which flow into the jejunum and further digested and absorbed into the brush border membrane.

Various proteolytic enzymes such as aminopeptidases and dipeptidases are produced by enterocytes in the brush border membrane, all of which are responsible for the final digestion of short-chain polypeptides (Pond et al., 2005). Most aminopeptidases from enterocytes belong to exopeptidase including aminopeptidase A, aminopeptidase N, and dipeptidyl-peptidase IV. Aminopeptidase A, generally referred to as glutamyl aminopeptidase, hydrolyzes Asp residues at the amino terminal end of peptide chains (Wang et al., 2013). Aminopeptidase N specifically cleaves the amino terminal end of peptide chains containing neutral AA residues (Turner, 2013). While both aminopeptidases A and N liberate free AA from peptide chains, dipeptidyl-peptidase IV catalyzes the hydrolysis of the carboxyl side of Pro at the second to last amino terminal end and liberates the dipeptide with Pro located at the carboxyl end (Misumi and Ikehara, 2013). Dipeptidyl-peptidase IV also acts on the carboxyl side of Ala and hydroxyproline, but the activity is lower than the residues with Pro (Misumi and Ikehara, 2013). Membrane dipeptidases produced by enterocytes specifically hydrolyze dipeptides to free AA regardless of the component of AA in dipeptides (Hooper, 2013).

1.2.3 Absorption of Amino Acids in the Gastrointestinal Tract

After several steps of protein digestion in the GIT, the final products of protein digestion, di- and tripeptides and free AA, in the lumen can be absorbed by either transcellular or paracellular transport (Trottier and Manjarín, 2012). The majority of AA absorption is through transcellular transport which includes simple diffusion, facilitated diffusion by transporter proteins, and active transport using energy-dependent transporter proteins (Trottier and Manjarín, 2012). Simple diffusion of free AA depends on the solubility of AA through the phospholipid bilayer membrane of enterocytes and concentration gradient of AA in enterocytes, whereas transporter-mediated absorption of free AA depends on the substrate specificity of transporter proteins which is usually driven by polarity of AA (Bröer, 2008; Trottier and Manjarín, 2012).

Most of energy-dependent transporter proteins involve an electrochemical gradient of Na^+ (Bröer, 2008). Electrochemical gradient of Na^+ toward cytosol is created by the Na^+-K^+ pump

which extrudes 3 Na^+ ions to the luminal area with the uptake of 2 K^+ ions using 1 molecule of adenosine triphosphate (Voet et al., 2008). The extrusion of Na^+ ions creates the local electrochemical gradient and provides Na^+ to Na^+-dependent transport systems which transport 1 molecule of free AA together with 1 molecule of Na^+ ion (Bröer, 2008). Major Na^+-dependent transport systems found in the brush border membrane of enterocytes and their substrates are as follows: system A, Gly, Pro, Ala, Ser, Cys, Gln, Asn, His, and Met; system ASC, Ala, Ser, Cys, Thr, and Gln; system B^0, neutral AA; system $B^{0,+}$, neutral and basic AA; system IMINO, Pro; system X^-_{AG}, acidic AA (Bröer, 2008). System $B^{0,+}$ and IMINO require electrochemical gradient of Cl^-, which is absorbed together with Na^+ and free AA (Bröer, 2008). On the other hand, system X^-_{AG} extrudes one molecule of K^+ ion when absorbing Na^+ and free AA (Bröer, 2008).

Similar to simple diffusion, facilitated diffusion depends on the concentration gradient of AA in enterocytes; however, it involves the transporter proteins which are driven by substrate specificity. System $b^{0,+}$, which is the major transport system for basic AA, is an antiporter which exchanges neutral AA in the cytosol for basic AA in the lumen (Bröer, 2008). System x^-_c also transports Glu by the extrusion of cystine to the lumen (Bröer, 2008). On the other hand, system PAT transports Pro, Gly, and Ala together with H^+ by negatively charged membrane potential (Bröer, 2008). System y^+ is an uniport that transports Arg, Lys, and His (Bröer, 2008).

Amino acids absorbed through the brush border membrane can be directly used by enterocytes or transported to the extracellular matrix through the basolateral membrane. Similar to the transport systems in the brush border membrane, free AA in the cytosol can be transported to the extracellular matrix via simple diffusion and transporter proteins. System T is found in the basolateral membrane which is a uniport for Phe, Tyr, and Trp by the concentration gradient (Bröer, 2008). System X^-_{AG} and y^+ are also found in the basolateral membrane which perform the same transport as their counterparts in the brush border membrane (Bröer, 2008). In addition, system y^+_L is responsible for the extrusion of basic AA by the uptake of neutral AA in the extracellular matrix (Bröer, 2008). On the other hand, unlike other transporter proteins located in the basolateral membrane, system L transports neutral AA except Pro from the circulation by the extrusion of Cys from the cytosol (Bröer, 2008).

Di- or tripeptides in the lumen are subject to digestion by membrane peptidases. However, depending on the composition of AA in peptides, several di- or tripeptides carry low affinity to membrane peptidase which can be directly absorbed by peptide transport system, PepT1 (Trottier

and Manjarín, 2012). System PepT1 is exclusively located in the brush border membrane and transports peptides by the gradient of H^+ (Trottier and Manjarín, 2012). Absorbed peptides can be digested to free AA in the cytosol or transported to the extracellular matrix, although the transport mechanism of peptides through the basolateral membrane has not been clearly elucidated (Trottier and Manjarín, 2012).

1.2.4 Metabolism of Amino Acids

Most AA absorbed after the digestion of dietary proteins are used for the synthesis of proteins required for various biological functions and growth of monogastric animals. However, before AA are transported to the organs, AA can be immediately metabolized in tissues of the GIT. Absorbed Glu and Gln are mostly metabolized and used as energy sources in enterocytes (Burrin and Stoll, 2009). When monogastric animals are under nutritionally challenging environments, additional supplementation of Glu or Gln may improve gut health and mitigate the impairment of growth performance (Bortoluzzi et al., 2018; Hou and Wu, 2018).

Majority of AA are used for the synthesis of skeletal muscle protein for growth of monogastric animals. Muscle protein synthesis is mediated by the insulin signaling pathway toward the mechanistic target of rapamycin C1 (**mTORC1**; Suryawan et al., 2011). Insulin secreted by increased glucose concentration in the circulation activates protein kinase B, which stimulates mTORC1 and consequently initiates the protein synthesis in the ribosome (Suryawan et al., 2011). In addition, previous studies reported that Leu may activate mTOR and stimulate protein synthesis in the skeletal muscle in pigs (Suryawan et al., 2011) and broiler chickens (Ospina-Rojas et al., 2019). Activated mTORC1 stimulates the ribosome to synthesize proteins; however, protein synthesis does not occur if all required AA are not present. That is, even if one AA is limited but the other AA are sufficient in the pool, ribosomes cannot synthesize complete protein. The AA which limits the overall protein synthesis is named as limiting AA (NRC, 2012). Therefore, if a diet contains sufficient AA except for one AA, which is below the requirement, remaining AA are deaminated and wasted.

Several AA are used for the synthesis of specific compounds required for maintenance of monogastric animals. Arginine is involved in the synthesis of urea which is the compound for excessive N excretion in pigs (Voet et al., 2008). Tryptophan is used for the synthesis of serotonin in the brain which regulates the appetite (Le Floc'h and Seve, 2007). In addition, Met produces S-

adenosylmethionine as the metabolic intermediate which acts as a major S donor, and Cys is used for the synthesis of glutathione which is an antioxidant in the body (Brosnan and Brosnan, 2006).

1.2.5 Requirements for Amino Acids

In order to maximize the productivity of monogastric animals, it is necessary to accurately estimate the amount of dietary AA required to maintain health and optimal growth. In both broiler chickens and pigs, the requirements for AA has been generally determined by feeding experimental diets containing graded concentrations of AA and measuring the growth performance. Experimental diets should contain at least 4 levels of AA from deficient to excessive concentrations for the broken-line or quadratic regression analysis, and the average daily gain, gain-to-feed ratio, and concentration of blood urea N can be used as responses (Baker, 1986).

Although direct measurement of AA requirements gives accurate concentration of dietary AA for animals used in experiments, there is a limitation in terms of implication of determined AA requirements in commercial farms due to the variations in genetic potential and housing environment. Therefore, modeling approach has been used to estimate the AA requirements of monogastric animals. In modeling approach for pigs, the requirements for AA is estimated by two major functions: maintenance and body protein deposition (van Milgen et al., 2008; NRC, 2012). The amount of AA required for maintenance is estimated based on the basal endogenous losses in the GIT and integument losses from skin and hair. On the other hand, the amount of AA required for body protein deposition is estimated by the stochastic models for body protein deposition including various factors such as physiological states, gender, genetic potential, or dietary characteristics.

In broiler chickens, however, extensive review to generate the prediction models for AA requirements is limited, although several commercial models have been developed for general production (Oviedo-Rondón, 2015). Sakomura et al. (2015) reported the factorial models to estimate the requirements for Lys, Met + Cys, Thr, and Val in male broiler chickens. In the models suggested in Sakomura et al. (2015), the AA requirements are estimated by two components similar to the models for pigs (i.e., maintenance and body protein deposition). However, the AA requirements for maintenance are estimated by the fixed coefficient relative to metabolic BW which were determined by the extrapolation of linear regression models conducted with N balance

study. In addition, the AA requirements for body protein deposition are estimated by the linear regression models between the deposition of AA and BW gain.

Protein quality of feed ingredients and diets have been generally evaluated by the ideal AA patterns, which is the ratio of the requirements for indispensable AA relative to that of Lys (Baker, 2003; NRC, 2012). Lysine has been used as a standard AA for ideal AA patterns because it is generally a first-limiting AA in commercial corn-SBM-based diets for pigs, although Met is often considered as a first-limiting AA in commercial diets for broiler chickens. In addition, together with Thr, de novo synthesis of Lys does not occur in monogastric animals, and therefore, Lys is completely indispensable AA (NRC, 2012). Moreover, Lys is almost exclusively used for body protein deposition (Möhn et al., 2000). Ideal AA patterns for broiler chickens and growing-finishing pigs suggested in previous studies are provided in Table 1.2. As AA requirements changes with BW of pigs, the ratio of indispensable AA to Lys also slightly changes. Therefore, target BW of animals needs to be considered when evaluating protein quality in feed ingredients using ideal AA patterns.

1.3 Determination of Amino Acid Availability

Availability of AA varies among feed ingredients, and it may be also affected by species of monogastric animals. Therefore, an accurate determination of AA availability with reliable methods is necessary for the optimal supply of AA to monogastric animals required for their maintenance and growth. In addition, various factors affecting digestibility of AA need to be assessed for an accurate evaluation of protein quality in feed ingredients. Basic concepts to describe the availability of AA are similar between broiler chickens and pigs, but there are several differences in terms of methodology.

1.3.1 Relative bioavailability of Amino Acids

Utilization of dietary AA by monogastric animals is often determined by the bioavailability of AA in test ingredients relative to standard ingredients. Relative bioavailability of AA is generally determined by the slope-ratio assay, which enables estimate of the proportion of the slope generated by responses from AA in test ingredients to the slope generated by responses from AA in standard ingredients (Batterham, 1992). Experimental diets should be prepared to be

deficient in AA of interest but not in other AA, and the concentration of AA of interest needs to gradually increase by either test ingredients or standard ingredients. The concentration of nutrients, other than AA of interest, and energy should be consistent in all experimental diets to ensure that responses such as growth performance or N retention is resulted from AA of interest. Moreover, results of experiments need to satisfy the 3 assumptions of slope-ratio assay: 1) linearity of responses; 2) common intercept for linear models from test ingredient and standard ingredient; 3) responses at 0 (i.e., blanks) for common intercepts (Littell et al., 1997). However, responses can be adjusted by appropriate statistical models if they do not fit into those assumptions.

Relative bioavailability of AA includes the overall results of a certain AA beyond digestion and absorption (Stein et al., 2007). However, because relative bioavailability of AA indicates the relative value to standard ingredients, there is a limitation in implication of this value in diet formulation. In addition, nutrient composition in experimental diets other than AA of interest may affect the responses, leading to a large variation among results (NRC, 2012). Studies have been conducted to determine the relative bioavailability of AA to compare the 2 different sources of crystalline AA in both broiler chickens and pigs (Shen et al., 2014; Kong et al., 2016; Wang et al., 2019).

1.3.2 Digestibility of Amino Acids

In both broiler chickens and pigs, nutritional values in feed ingredients and diets have been generally determined by digestibility of nutrients. Digestibility of nutrients represents the proportion of nutrients in feed ingredients or diets which can be digested and absorbed by monogastric animals. Indeed, digestibility values are determined by 'indigestibility' of nutrients which is calculated by the difference between the intake and excretion of nutrients (Adeola, 2001).

1.3.2.1 Methods to Determine Digestibility of Nutrients

Digestibility of nutrients can be directly measured if quantitative collection of feces or excreta is possible, which is referred to as the total collection method (Kong and Adeola, 2014). In the total collection method, it is necessary to accurately record the weight of feed intake and excretion of experimental animals (Adeola, 2001). Therefore, animals are generally housed

individually to measure the individual feed intake and separate the excretion of feces, urine, or excreta from those excreted by other animals.

In the total collection method with pigs, feed intake is often restricted by offering a calculated daily feed allowance to avoid the waste of feed and feed refusal which hinder the accurate measurement of feed intake. Daily feed allowance can be prepared with 2.5 to 3.5 times the estimated metabolizable energy requirement (e.g., 197 kcal/kg $BW^{0.60}$; NRC, 2012) or with 2.7 to 4% of BW (Adeola, 2001). Daily feed allowance may be adjusted during the adaptation period to ensure that diets are completely consumed when offered to pigs during the collection period. After 3 to 7 d of adaptation period, feces originating from a given diet is quantitatively collected for 4 to 6 d of collection period (Adeola, 2001). For the quantitative collection of feces, the markers, indigestible compounds that change the color of feces, should be included in the first meal of the collection period and the first meal immediately after the collection period which enables the collection of feces originating from experimental diets offered during the collection period. Basic assumption of using the marker is that it moves along with the digesta in the GIT without being diffused to digesta originating from meals which do not contain the marker (Adeola, 2001). Compounds that have been used as markers include chromic oxide, ferric oxide, and indigo carmine (Adeola, 2001).

In the total collection method with broiler chickens, precision-fed roosters or cockerels are generally used to determine the digestibility of nutrients in feed ingredients (Kong and Adeola, 2014). Before feeding test ingredients or experimental diets, birds are fasted for 24 to 48 hr to empty the GIT. Then birds are fed test ingredients by inserting a feeding tube via the beak to the crop, and the quantitative collection of excreta is conducted for 48 hr. The assumption for the precision-fed assay is that collected excreta originates from force-fed test ingredients or experimental diets.

Using the weight of feed intake, feces or excreta, and the analyzed concentration of the nutrient of interest in diets and feces or excreta, digestibility of a nutrient is calculated by the following equation (Kong and Adeola, 2014):

$$\text{Digestibility of nutrient (\%)} = \left[\frac{(FI \times N_I) - (FO \times N_O)}{FI \times N_I}\right] \times 100 \quad [1],$$

where FI and FO are the weight of feed intake and feces or excreta output [kg dry matter (**DM**)], and N_I and N_O are the concentration of nutrient in feed and feces or excreta (g/kg DM).

Because the quantitative collection of feces or excreta is available by individually housing animals in specialized crates or pens, it has been generally used to determine the total tract digestibility of nutrients which represents the digestible and absorbable nutrients throughout the overall GIT. In broiler chickens, excreta contains both feces and urine. Therefore, the value determined by the total collection method is considered as total tract metabolizability or retention of nutrients.

The total collection method is available only when the quantitative collection of feces or excreta from animals is available. However, digestibility of nutrients can be measured even if the quantitative collection of nutrients is not available. The index method enables calculation of the digestibility of nutrients using the indigestible index compound added in experimental diets. Unlike the marker used for the total collection method, indigestible index compounds do not indicate the initiation or termination of collection by changing color of feces or excreta but indicates the ratio between feed intake and excretion of feces or excreta. Indigestible index materials which have been generally used in digestibility trials include chromic oxide, titanium dioxide, and acid insoluble ash (Wang et al., 2017). Basic assumptions for the index method is as follows: 1) the indigestible index compound is completely indigestible and not absorbed or retained in the GIT; 2) the indigestible index compound is regularly excreted by feces or excreta together with undigested nutrients; 3) the indigestible index compound is uniformly distributed in diets and feces or excreta (Kong and Adeola, 2014). Therefore, it is assumed that the indigestible index material intake equals the amount of indigestible index materials excreted, which can be described as the following equations:

$$FI \times X_I = FO \times X_O \quad [2];$$

$$\frac{FO}{FI} = \frac{X_I}{X_O} \quad [3],$$

where X_I and X_O are the concentration of indigestible index (g/kg DM). On the other hand, Equation 1 for the total collection method can be rearranged as follows:

$$\text{Digestibility of nutrient (\%)} = \left[1 - \frac{FO \times N_O}{FI \times N_I}\right] \times 100 = \left[1 - \left(\frac{FO}{FI} \times \frac{N_O}{N_I}\right)\right] \times 100 \quad [4].$$

Incorporating Equation 3 into Equation 4 derives the formula to calculate the digestibility of nutrients using the index method (Kong and Adeola, 2014):

$$\text{Digestibility of nutrient (\%)} = \left[1 - \left(\frac{X_I}{X_O} \times \frac{N_O}{N_I}\right)\right] \times 100 \quad [5].$$

The index method has been generally used to determine the ileal digestibility of nutrients because quantitative collection of ileal digesta is practically difficult, although several techniques enable the total collection of ileal digesta. In addition, the index method has been used to determine the total tract digestibility of nutrients when monogastric animals are housed in groups, thus, separate collection of feces or excreta from individual animals is limited. While an accurate record of feed intake and weight of feces or excreta is fundamental in the total collection method, chemical analysis of indigestible index material is critical in the index method, which are susceptible to variation due to the low concentration of indigestible index material in experimental diets and feces or excreta (Adeola, 2001).

1.3.2.2 Total Tract and Ileal Digestibility of Amino Acids

Among essential nutrients required for broiler chickens and pigs, digestibility of AA has been determined by ileal digestibility rather than total tract digestibility due to the following reasons. First, most of undigested AA are subject to microbial fermentation in the hindgut of monogastric animals. Various bacteria reside in the ceca of broiler chickens and large intestine of pigs, and proteins and AA are required to sustain the proliferation of microbiome. After proteins and AA in digesta are used by bacteria, ammonia and amines are excreted as metabolic end products of microbial fermentation (Just et al., 1981). Second, the analyzed concentration of AA in feces or excreta includes AA originating from gut microbes excreted together with undigested AA. Microbial population in the hindgut continuously changes depending on availability of nutrients and environment of the lumen. Therefore, a decrease in microbial population results in death of microbial cells which can be either utilized by other gut microbes or excreted via feces or excreta. Nitrogen content originating from gut microbes account for 62 to 76% of total N content in feces of pigs (NRC, 2012). Third, the majority of AA absorption occurs in the small intestine of both broiler chickens and pigs. As discussed earlier, enzymatic digestion of proteins mainly occurs in the foregut and small intestine. In addition, free AA and peptides liberated by proteolytic digestion are absorbed by enterocytes in the small intestine via diffusion or transporter proteins. Sauer et al. (1980) reported that the mean difference between apparent ileal and total tract digestibility of CP and AA was 7.6% units. Overall, ileal digestibility of AA is preferred to express the availability of AA compared to total tract digestibility of AA where availability of AA may be

biased by microbial fermentation of the hindgut with negligible absorption of AA (Sauer and Ozimek, 1986).

1.3.2.3 Experimental Diets for Digestibility of Amino Acids

Determination of AA digestibility in feed ingredients depends on the preparation of experimental diets. Digestibility of AA in test ingredients can be directly measured by preparing experimental diets containing test ingredients as the sole source of N, which is referred to as the direct method (Kong and Adeola, 2014). In the direct method, nutrients other than AA are supplied by purified ingredients such as cornstarch, dextrose, sucrose, limestone, or mono- or dicalcium phosphate. The direct method has been generally used in studies for determination of digestibility of AA in feed ingredients due to the ease of experimental diet preparation and calculation. However, it is hardly possible to provide the AA requirements of monogastric animals using a single test ingredient. In addition, most feed ingredients contain imbalanced AA contents required for monogastric animals which may hinder the digestibility of AA. Therefore, insufficient and imbalanced AA concentrations in experimental diets may negatively affect and underestimate the digestibility of AA compared to the actual potential of monogastric animals to digest and absorb AA in test ingredients.

The difference method has often been used when test ingredients cannot provide sufficient nutrients of interest or high concentration of test ingredients in experimental diets causes adverse effects on animals such as reduced palatability or toxicity by antinutritional factors (Kong and Adeola, 2014). In the difference method, a reference diet is prepared with typical feed ingredients such as corn or SBM and contains adequate concentrations of nutrients. Test ingredients are added into the reference diet, and digestibility of nutrients of interest is calculated by the difference between digestibility values for experimental diets and the reference diet (Kong and Adeola, 2014). In studies for digestibility of AA, although using the difference method can prevent the potential negative effects of insufficient and imbalanced AA concentrations in diets, the difference method is rarely used except for specific feed ingredients due to the complex methodology and calculations as well as high susceptibility to variation among observations.

1.3.3 Methods to Determine Ileal Digestibility of Amino Acids

Unlike the collection of feces or excreta, the collection of ileal digesta often involves invasive techniques which may interrupt the normal physiological condition of monogastric animals. Therefore, sophisticated surgical techniques have been developed to minimize the negative effects caused by the collection of ileal digesta samples. In addition, numerous studies have been conducted to evaluate the accurate ileal digestibility of AA by correcting AA which do not originate from experimental diets (i.e., endogenous losses), leading to a development of terminologies to describe the ileal digestibility of AA.

1.3.3.1 Techniques to Determine Ileal Digestibility in Pigs

In studies to determine ileal digestibility of AA in pigs, surgical insertion of T-cannula has been widely used which enables the collection of ileal digesta for a certain period of time (Sauer and Ozimek, 1986; Stein et al., 1998; Dilger et al., 2004). Before the surgery, pigs are fasted overnight to empty the small intestine and prevent infection by remaining digesta during the surgery. Pigs are anesthetized by injection of appropriate doses based on BW, and gas anesthesia is applied during the surgery. Then, the right posterior abdomen is incised through the peritoneum, and the distal ileum is longitudinally incised at 5 to 10 cm proximal to the ileocecal junction to insert the T-cannula. After insertion, the incision at the distal ileum is tightly sutured, and a puncture is made to exteriorize the barrel of the T-cannula. At last, the incisions on the peritoneum and abdomen are sutured and disinfected by antibiotic ointment. Before pigs are introduced to experiment, pigs are monitored and treated for 7 to 14 d as recovery period. During the recovery period, daily feed allowance is gradually increased to prevent the rupture of the incision area, and defecation is monitored which indicates the continuous flow of digesta in the GIT. The collection of ileal digesta during the experimental period is conducted by placing the plastic sample bags on the barrel of T-cannula. However, because ileal digesta can also flow into the cecum, total collection of ileal digesta is not possible using the T-cannula. Therefore, representativeness of collected ileal digesta samples is critical which may be controlled by frequency and duration of sample collection (Sauer and Ozimek, 1986).

While the quantitative collection of ileal digesta is not available in the technique using T-cannula, it is available in other techniques which also involve the surgical insertion of cannulas.

The technique using re-entrant cannula involves surgical insertion of proximal and distal cannulas at the distal ileum (Żebrowska and Buraczewski, 1998). Ileal digesta can be quantitatively collected via the proximal cannula, whereas ileal digesta flows through the distal cannula when ileal digesta samples are not collected. Because the re-entrant cannula technique requires transection of the distal ileum, the enteric nervous system is disrupted which controls the motility of digesta in the GIT (Sauer and Ozimek, 1986). Therefore, the collection of ileal digesta is often hindered by blockage of the cannula (Żebrowska and Buraczewski, 1998).

Ileo-rectal anastomosis is the technique in which the quantitative collection of ileal digesta is also available. In this technique, the distal ileum is surgically connected to the distal colon to bypass and isolate the large intestine (Green et al., 1987). One of the major defects in ileo-rectal anastomosis is that digestive physiology in the GIT changes after the surgery. For example, diets need to contain greater concentrations of minerals due to the absence of mineral absorption in the large intestine (Żebrowska and Buraczewski, 1998). In addition, the intake of water increases because the absorption of water in the GIT is limited without the large intestine (Żebrowska and Buraczewski, 1998).

Both re-entrant cannula technique and ileo-rectal anastomosis enable the quantitative collection of ileal digesta but require the transection of the intestine. However, the quantitative collection of ileal digesta without transection of the intestine can be achieved by surgical insertion of the post valve T-cecum cannula (**PVTC**). The cannula surgically fitted at the cecum extrudes ileocecal junction into the barrel of cannula when the cannula is open, which prevents the flow of ileal digesta to the cecum and allows quantitative collection of ileal digesta (van Leeuwen et al., 1991). On the other hand, when the cannula is closed, ileal digesta flows into the cecum and do not disturb the overall flow of digesta in the GIT (van Leeuwen et al., 1991).

One of the major advantages of the T-cannula technique is that the surgical procedure is relatively simple compared to the other techniques (Nyachoti et al., 1997). In addition, the T-cannula technique does not involve the transection of the intestine which disrupts the motility of digesta in the GIT. Although the collection of representative ileal digesta samples and reliability on chemical analysis of indigestible index markers are major issues in T-cannula technique, pigs can maintain the normal physiological condition after the surgery which is required for an accurate determination of digestibility of AA.

1.3.3.2 Techniques to Determine Ileal Digestibility in Broiler Chickens

Similar to the T-cannula technique in pigs, the technique which involves the surgical insertion of cannulas is also available in broiler chickens (Gurnsey et al., 1985); however, use of this technique is limited due to the complex surgical procedures and blockage of the cannula (Parsons, 2002). On the other hand, cecectomy has been performed to determine the ileal digestibility of AA which is relatively simpler than the surgical procedures for cannulation (Parsons, 2002). In this technique, the ceca of adult roosters or cockerels are surgically removed which allows ileal digesta to bypass the ceca and directly flow into the cloaca. Previous studies have been used cecectomized roosters or cockerels with precision-fed assay in which the quantitative collection of ileal digesta is possible (Parsons, 1985; Adedokun et al., 2009). However, one of the limitations of using precision-fed cecectomized roosters or cockerels is the potential effects of force-feeding which is different from normal feed intake of broiler chickens (Parsons, 2002). In addition, removed ceca may interrupt the normal physiological condition of birds which may negatively affect the digestibility of AA.

Instead of the invasive techniques, ileal digesta can be collected after euthanizing broiler chickens (Kong and Adeola, 2014). A group of broiler chickens are fed experimental diets for 4 to 5 d, and then birds are euthanized and dissected for the collection of ileal digesta, which is generally collected from distal portion of the ileum (i.e., from Meckel's diverticulum to ileocecal junction). Approximately distal two-thirds of the ileum is subject to the ileal digesta collection by flushing distilled water because most of AA absorption is completed before the ileum and sufficient sample is required for the chemical analysis (Kadim and Moughan, 1997). In general, the index method is used together with the euthanizing technique due to the limitation of quantitative ileal digesta collection.

1.3.3.3 Endogenous Losses of Amino Acids

In both broiler chickens and pigs, ileal digesta not only contains undigested dietary proteins and AA but also contains proteins and AA originating from the GIT and microbiome, which is considered as ileal endogenous losses (Stein et al., 2007; Adeola et al., 2016). Nyachoti et al. (1997) reported that ileal endogenous losses of AA originate from endogenous secretion from the digestive organs which are not reabsorbed, mucus, sloughed epithelial cells, and bacteria in the

small intestine. Ileal endogenous losses of AA can be divided into basal and specific ileal endogenous losses depending on the consideration of diet-specific factors (Adeola et al., 2016). Basal ileal endogenous losses (**BEL**) of AA are considered as the minimum losses of AA from the physical flow of digesta but not by diet-specific factors (Stein et al., 2007; Adeola et al., 2016). On the other hand, specific ileal endogenous losses of AA are considered as the losses of AA due to diet-specific factors such as ingredient and nutrient composition of diets or antinutritional factors (Stein et al., 2007; Adeola et al., 2016). Therefore, if the concentration of test ingredient in experimental diets increases, the BEL of AA are not affected, whereas the specific ileal endogenous losses of AA gradually increases (Stein et al., 2007).

Various methods have been used to determine the BEL of AA in both broiler chickens and pigs. Feeding a N-free diet (**NFD**) has been commonly used to determine the BEL of AA due to its simplicity (Kong and Adeola, 2014). The NFD is prepared based on purified carbohydrate sources such as cornstarch or dextrose without any ingredient which contains proteins or AA. However, feeding NFD has been criticized due to the deficiency of AA in animals which affects the physiological condition of animals (Adeola et al., 2016). In addition, the overall BEL of AA may be underestimated due to the absence of dietary proteins which potentially stimulate the endogenous enzyme secretions (Stein et al., 2007). To overcome the potential negative effects caused by feeding NFD, feeding a diet containing low concentration of casein has been proposed based on the assumption that added casein is completely digested and absorbed (Nyachoti et al., 1997). However, this assumption remains unclear, and consequently the BEL of AA may be overestimated due to the undigested proteins and AA from casein (Nyachoti et al., 1997). Adedokun et al. (2007) reported that the BEL of AA in broiler chickens linearly increased with increasing concentration of casein from 0 to 150 g/kg. In addition, Zhang et al. (2002) reported that the BEL of most AA in pigs fed NFD were less than those in pigs fed the diet containing 50 g/kg casein. On the other hand, undigested proteins can be separated from the BEL of AA in the method using enzymatically-hydrolyzed casein (Darragh et al., 1990). In this method, animals are fed a diet containing enzymatically-hydrolyzed casein which consists of low-molecular weight peptides, and proteins in ileal digesta are separated by size to differentiate the BEL of AA and remaining undigested peptides. However, the BEL of AA may be overestimated because low-molecular weight peptides may stimulate the endogenous proteolytic enzymes in the GIT (Golian

et al., 2008). When the index method is used together with the aforementioned methods, the BEL of AA is calculated by the following equations (Kong and Adeola, 2014):

$$\text{BEL of AA (g/kg DM intake)} = \left[\frac{FO \times N_O}{FI}\right] = \left[N_O \times \frac{FO}{FI}\right] = \left[N_O \times \frac{X_I}{X_O}\right] \quad [6].$$

In the technique using precision-fed cecectomized roosters or cockerels, the BEL of AA is estimated by collecting excreta from fasted birds (Parsons, 1985). However, this method may underestimate the BEL of AA because of the absence of feed intake (Adeola et al., 2016). Moreover, Adedokun et al. (2007) reported that the BEL of AA in broiler chickens were affected by the age of broiler chickens, which may imply that the BEL of AA in adult roosters and cockerels are not applicable for young broiler chickens.

Several methods have been studied to estimate the total ileal endogenous losses (**TEL**) of AA, which is the sum of BEL and specific ileal endogenous losses of AA. The isotope dilution method involves animals continuously infused by ^{15}N-AA, which labels the N pool of animals and enables the separation of TEL of N from undigested N in ileal digesta (de Lange et al., 1989). In addition, in the homoarginine method, animals are fed experimental diets processed by guanidination of Lys to homoarginine which allows the determination of the TEL of Lys (Nyachoti et al., 1997). Although the measurement of TEL of AA contributes to determination of absolute digestibility of AA in feed ingredients and diets, both isotope dilution and homoarginine methods require specialized analytical tools and laborious procedures (Stein et al., 2007).

Each method to determine either BEL or TEL of AA carries limitations or specific assumptions which have not been completely verified. As a result, despite concerns regarding the deficiency of AA in animals, feeding NFD has been generally used to estimate the BEL of AA and subsequently calculate the SID of AA (Stein et al., 2007; Kong and Adeola, 2014). The mean BEL of AA in broiler chickens and pigs suggested in previous studies which summarized the values reported in published articles are presented in Table 1.3.

1.3.3.4 Terminologies for Ileal Digestibility of Amino Acids

Ileal digestibility of AA without the correction of ileal endogenous losses of AA is defined as the apparent ileal digestibility (**AID**; Stein et al., 2007). Because collected ileal digesta samples contain AA originating from both undigested proteins and ileal endogenous losses, digestibility values are affected by the concentration of CP in experimental diets (Fan et al., 1994). In collected

ileal digesta samples, the proportion of AA originating from ileal endogenous losses decreases with increasing proportion of AA originating from dietary undigested proteins and AA. Therefore, if the concentration of CP in experimental diets gradually increases, the AID of AA quadratically increases and reaches plateau (Fan et al., 1994). However, it should be noted that the maximum AID of AA (i.e., values at the plateau) which is not affected by dietary CP concentration still underestimates the digestibility of AA due to the remaining endogenous losses of AA in ileal digesta.

Definitions of ileal digestibility of AA with the correction of ileal endogenous losses depend on the component of ileal endogenous losses used in calculations. The SID of AA represents the ileal digestibility corrected for the BEL of AA, whereas the true ileal digestibility of AA represents the ileal digestibility corrected for the TEL of AA (Stein et al., 2007). In the calculation of SID of AA, the BEL of AA is deducted from the total AA in ileal digesta based on the following equation:

$$\text{SID of AA (\%)} = \left[\frac{(FI\times N_I) - \{(FO\times N_O) - (FI\times BEL)\}}{FI\times N_I}\right]\times 100 \quad [7].$$

This equation can be simplified by rearrangement and applying AID values as follows (Kong and Adeola, 2014):

$$\text{SID of AA (\%)} = \left[\frac{(FI\times N_I) - (FO\times N_O)}{FI\times N_I} + \frac{BEL}{N_I}\right]\times 100 = \text{AID} + \left[\frac{BEL}{N_I}\times 100\right] \quad [8].$$

Equations for the SID of AA can be also applied to calculate the true ileal digestibility of AA by replacing the BEL with TEL of AA.

Due to the practical limitations in the determination of TEL of AA, the SID of AA has been widely used to evaluate the availability of AA in feed ingredients and diets (Kong and Adeola, 2014). Moreover, previous studies have verified that the SID of most AA in test ingredients are additive in mixed diets for both broiler chickens and pigs (Wang et al., 2018; Osho et al., 2019). While the AID of AA in feed ingredients are not additive in mixed diets due to the influence of dietary CP concentrations, the SID of AA in feed ingredients are additive because the BEL of AA in ileal digesta is adjusted relative to DM intake of animals. Due to its additivity and consistency among experiments, the SID of AA is also used to describe the AA requirements of pigs (NRC, 2012). In broiler chickens, however, few studies have been conducted to estimate the requirements for standardized ileal digestible AA (Zaboli et al., 2011; Lee et al., 2018). Extensive review to

summarize the AA requirements as well as terminologies for digestibility of AA for broiler chickens are required.

1.3.4 Factors Affecting Digestibility of Amino Acids

Various techniques have been used to collect ileal digesta samples from monogastric animals, and each technique carries specific assumptions which generally influence the variation among results. Consequently, the digestibility of AA in test ingredients may be affected by the techniques used to determine. In pigs, Köhler et al. (1990) reported that the AID of N in the corn-wheat-SBM-based diet and pectin-rich diet were not different among the PVTC, T-cannula, and re-entrant cannula techniques; however, the AID of N in the fiber-rich diet and cornstarch-wheatstarch-based diet determined by the PVTC technique was greater than the values determined by the re-entrant cannula technique. In broiler chickens, Adedokun et al. (2009) reported that the SID of most AA in distillers' dried grains with solubles and corn were not affected by the techniques to determine digestibility of AA; however, broiler chickens fed diets containing canola meal, SBM, and meat and bone meal and euthanized for ileal digesta collection had greater SID of AA compared to precision-fed cecectomized roosters. Therefore, to mitigate the variation in results among studies, it is necessary to use consistent techniques to determine the digestibility of AA for appropriate interpretation of results.

Digestibility of AA determined by the index method relies on the indigestible index compound used in experimental diets. Therefore, the type of indigestible index material may affect the digestibility of AA. Previous study conducted by Wang and Adeola (2018) revealed that in pigs fed corn-SBM-based diets, the AID of N determined by titanium dioxide as the indigestible index material was greater than the value determined by chromic oxide. However, Wang et al. (2018) reported that the AID or SID of AA in experimental diets mainly based on cornstarch was not affected by the type of indigestible index compound when titanium dioxide and chromic oxide were used. Therefore, it may be concluded that the type of indigestible index compound affects the digestibility of AA in corn-SBM-based diets, but not in semi-purified experimental diets (Wang and Adeola, 2018).

Digestibility of AA can be affected by various diet-specific factors such as the concentration of dietary fiber, antinutritional factors, or processing of diets. Dietary fiber influences physiological functions of the GIT such as motility, pancreatic enzyme secretions, or

synthesis and secretion of mucus (Montagne et al., 2003), all of which are directly related to the digestion and absorption of AA. In addition, enzymatic digestion of dietary proteins and AA may be hindered by dietary fiber because it is the major component of the plant cell wall which encapsulates nutrients in plant cells (Adeola and Cowieson, 2011). Dilger et al. (2004) found that the SID of several AA in SBM linearly decreased with increasing concentration of soyhulls in experimental diets fed to pigs. Spindler et al. (2016) also reported the negative relationship between the SID of AA in pigs and the concentration of neutral detergent fiber in barley.

Several feed ingredients contain antinutritional factors such as trypsin inhibitors in SBM or glucosinolates in rapeseed meal (Chiba, 2001). Trypsin inhibitors in soybean and SBM can bind to endogenous trypsin secreted by pancreas and interfere with the digestion of dietary proteins. Palliyeguru et al. (2011) reported that increasing trypsin inhibitor activity in corn-SBM-based diets linearly decreased the AID of CP in broiler chickens. To reduce the activity of trypsin inhibitors, heat treatment has been applied in the production of full-fat soybean and SBM for monogastric animals (Chiba, 2001). In rapeseed meal, glucosinolates does not carry antinutritional effects; however, the hydrolysis of glucosinolates by myrosinase produces toxic compounds which mainly cause goiter or thyroid dysfunction (Tripathi and Mishra, 2007). Glucosinolates and myrosinase are separately stored in rapeseeds, but physical processing or mastication of animals ruptures the storage structures and causes the reaction between glucosinolates and myrosinase (Tripathi and Mishra, 2007). To mitigate the negative effects of glucosinolates, rapeseeds containing less than 30 μmol/g glucosinolates in the meal after oil extraction have been selected, which is referred to as canola seeds (Canola Council of Canada, 2015). In addition, heat treatment during the production of canola meal further reduces the negative effects of glucosinolates by inactivating myrosinase (Chiba, 2001). Therefore, heat treatment is necessary for processing of SBM and canola meal in order to reduce the negative effects of antinutritional factors. However, excessive heat treatment may denature the structure of proteins and eventually reduce the digestibility of AA, especially for Lys which binds to reducing sugars and form unreactive Lys in high temperatures (i.e., Maillard reaction; Kim et al., 2012). Previous studies have reported reduced SID of AA in pigs by excessive heat treatment for SBM (González-Vega et al., 2011) and canola meal (Almeida et al., 2014).

Even though digestibility of AA in plant feed ingredients are affected by various factors, it is relatively consistent among sources compared to feed ingredients originating from animals.

Digestibility of AA in feed ingredients originating from animals varies among sources due to the large variation in processing conditions among rendering plants (Hicks and Verbeek, 2016). Differences in processing conditions such as byproduct components in final products, pressure, temperature, or time of heat treatment potentially affect the digestibility of AA. Ravindran et al. (2002) reported that the CV of AA concentrations in 19 sources of meat and bone meal ranged from 9.1% for Pro to 33.5% for Cys and that the AID of most AA were negatively correlated with the concentration of ash in broiler chickens. In addition, Shirley and Parsons (2000) found that the SID of AA in meat and bone meal was reduced when pressure during processing increased.

1.4 Summary

Amino acids are required for maintenance of normal physiological functions and growth of monogastric animals. Each AA carries its own specific characteristics, and classification of AA by nutritional necessity has been generally applied in monogastric nutrition. Indispensable AA include the AA which need to be provided by dietary proteins, whereas dispensable AA include the AA which can be sufficiently synthesized in the body of monogastric animals. Dietary proteins are mainly digested in the foregut and small intestine. However, there are distinct differences in the foregut between broiler chickens and pigs. The foregut of broiler chickens consists of the crop, proventriculus, and gizzard, whereas the stomach is the sole digestive organ in the foregut of pigs which is responsible for proteolytic digestion. After proteolytic digestion in the foregut, partially digested proteins and polypeptides are further digested in the small intestine by pancreatic enzymes and mucosal enzymes. Finally, free AA and di- and tripeptides, which are end products of proteolytic digestion, are absorbed into enterocytes. Absorbed AA can be either metabolized in enterocytes or transported to various organs and used for protein synthesis.

To maximize the growth performance of animals, diets need to be prepared to meet the requirements for AA. In both broiler chickens and pigs, the AA requirements can be estimated by the factorial models using the requirements for maintenance and body protein deposition as components. Based on the estimated AA requirements, ideal AA patterns can be calculated and used to evaluate the protein quality of feed ingredients and diets.

Availability of AA in feed ingredients and diets has been generally evaluated by the digestibility of AA. In addition, due to microbiome in the hindgut, ileal digestibility rather than total tract digestibility has been used to evaluate the digestibility of AA. As a result, various

techniques involving surgical procedures have been developed. In pigs, the T-cannula technique has been widely used due to its simplicity, although the collection of representative ileal digesta is controversial. In broiler chickens, euthanizing birds to collect ileal digesta samples may be preferred over using precision-fed cecectomized roosters or cockerels which involves peculiar and invasive feeding techniques.

Amino acids in collected ileal digesta consist of AA originating from undigested dietary proteins and AA originating from endogenous losses. Ileal endogenous losses of AA can be divided into the BEL and specific ileal endogenous losses. The BEL of AA represents the inevitable and minimal losses of AA from animals, whereas specific ileal endogenous losses of AA represents the ileal endogenous losses induced by diet-specific factors. The BEL of AA has been generally determined by feeding NFD due to its simplicity. On the other hand, determination of the TEL of AA requires specific analytical tools and laborious procedures.

Ileal digestibility of AA can be divided into apparent, standardized, and true ileal digestibility depending on the correction of ileal endogenous losses of AA. The AID of AA is calculated without the correction of the ileal endogenous losses of AA, whereas the SID and TID of AA are calculated by the correction of BEL and TEL of AA, respectively. Due to the limitations in determination of the TEL of AA and additivity of SID values in mixed diets, the SID of AA has been widely used to evaluate the availability of AA in feed ingredients and diets as well as to describe the AA requirements of animals. Digestibility of AA can be affected by various factors including techniques to collect ileal digesta, the type of indigestible index, and diet-specific factors such as the concentration of dietary fiber, antinutritional factors, or processing of feed ingredients.

Experimental diets to determine the ileal digestibility of AA in feed ingredients have been prepared mainly based on purified carbohydrates such as cornstarch or dextrose; however, semi-purified experimental diets may affect the ileal digestibility of AA due to deficient and imbalanced AA concentrations in experimental diets. In addition, previous studies have determined the effects of factors affecting digestibility of AA within broiler chickens or pigs. However, the effects of factors affecting digestibility of AA may be different between broiler chickens and pigs.

1.5 Objective

The objective of the studies conducted for this dissertation was to determine the effects of casein in experimental diets on SID of AA in corn distillers' dried grains with solubles and to

compare the SID of AA in feed ingredients, which include full-fat soybean, two SBM, peanut flour, full-fat canola seeds, canola meal, canola expellers, hydrolyzed feather meal, flash-dried poultry protein, poultry meal, and meat and bone meal, in broiler chickens and pigs.

1.6 Literature Cited

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590-2597. doi:10.3382/ps.2007-00096

Adedokun, S. A., P. Utterback, C. M. Parsons, O. Adeola, M. S. Lilburn, and T. J. Applegate. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350-358. doi:10.1080/00071660902951321

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903-916.

Adeola, O., and A. J. Cowieson. 2011. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189-3218. doi:10.2527/jas.2010-3715

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274-283. doi:10.1016/j.anifeedsci.2016.06.004

Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2014. Effects of heat treatment on the apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim. Feed Sci. Technol. 187:44-52. doi:10.1016/j.anifeedsci.2013.09.009

Auld, D. S. 2013. Carboxypeptidase A. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 1289-1301.

Avilés, F. X., and J. Vendrell. 2013. Carboxypeptidase B. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 1324-1329.

Baird Jr., T. T., and C. S. Craik. 2013. Trypsin. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 2594-2600.

Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. J. Nutr. 116:2339-2349. doi:10.1093/jn/116.12.2339

Baker, D. H. 2003. Ideal amino acid patterns for broiler chicks. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. 2nd ed. CAB International, Cambridge, MA. p. 223-235.

Batterham, E. S. 1992. Availability and utilization of amino acids for growing pigs. Nutr. Res. Rev. 5:1-18. doi:10.1079/NRR19920004

Bortoluzzi, C., S. J. Rochell, and T. J. Applegate. 2018. Threonine, arginine, and glutamine: Influences on intestinal physiology, immunology, and microbiology in broilers. Poult. Sci. 97:937-945. doi:10.3382/ps/pex394

Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. Physiol. Rev. 88:249-286. doi:10.1152/physrev.00018.2006

Brosnan, J. T., and M. E. Brosnan. 2006. The sulfur-containing amino acids: An overview. J. Nutr. 136:1636S-1640S. doi:10.1093/jn/136.6.1636S

Burrin, D. G., and B. Stoll. 2009. Metabolic fate and function of dietary glutamate in the gut. Am. J. Clin. Nutr. 90:850S-856S. doi:10.3945/ajcn.2009.27462Y

Canola Council of Canada. 2015. Canola meal feeding guide. 5th rev. ed. Canola Council of Canada, Winnipeg, Manitoba, Canada.

Chiba, L. I. 2001. Protein supplements. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 803-837.

Crévieu-Gabriel, I., J. Gomez, J. -P. Caffin, and B. Carré. 1999. Comparison of pig and chicken pepsins for protein hydrolysis. Reprod. Nutr. Dev. 39:443-454. doi:10.1051/rnd:19990404

Darragh, A. J., P. J. Moughan, and W. C. Smith. 1990. The effect of amino acid and peptide alimentation on the determination of endogenous amino acid flow at the terminal ileum of the rat. J. Sci. Food Agric. 51:47-56. doi:10.1002/jsfa.2740510106

de Lange, C. F. M., W. C. Sauer, and W. Souffrant. 1989. The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J. Anim. Sci. 67:755-762. doi:10.2527/jas1989.673755x

Denbow, D. M. 2000. Gastrointestinal anatomy and physiology. In: G. C. Whittow, editor, Sturkie's avian physiology. 5th ed. Academic Press, New York, NY. p. 299-325.

de Oliveira, E. B., and M. C. O. Salgado. 2013. Pancreatic elastases. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 2639-2645.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

D'Mello, J. P. F. 2003. Amino acids as multifunctional molecules. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. 2nd ed. CAB International, Cambridge, MA. p. 1-14.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: Effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859. doi:10.2527/1994.72112851x.

Golian, A., W. Guenter, D. Hoehler, H. Jahanian, and C. M. Nyachoti. 2008. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. Poult. Sci. 87:706-712. doi:10.3382/ps.2007-00330

González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci. 89:3617-3625. doi:10.2527/jas.2010-3465

Gráf, L., L. Szilágyi, and I. Venekei. 2013. Chymotrypsin. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 2626-2633.

Green, S., S. L. Bertrand, M. J. C. Duron, and R. A. Maillard. 1987. Digestibility of amino acids in maize, wheat and barley meal, measured in pigs with ileo-rectal anastomosis and isolation of the large intestine. J. Sci. Food Agric. 41:29-43. doi:10.1002/jsfa.2740410104

Gurnsey, M. P., K. A. C. James, and D. C. Johns. 1985. An improved method of ileal cannulation of adult cockerels. Res. Vet. Sci. 39:390-391. doi:10.1016/S0034-5288(18)31733-8

Hicks, T. M., and C. J. R. Verbeek. 2016. Meat industry protein by-products: Sources and characteristics. In: G. S. Dhillon, editor, Protein byproducts: Transformation from environmental burden into value-added products. Academic Press, London, UK. p. 37-61. doi:10.1016/B978-0-12-802391-4.00003-3

Hooper, N. M. 2013. Membrane dipeptidase. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 1670-1673.

Hou, Y., and G. Wu. 2018. L-Glutamate nutrition and metabolism in swine. Amino Acids 50:1497-1510. doi:10.1007/s00726-018-2634-3

Just, A., H. Jørgensen, and J. A. Fernández. 1981. The digestive capacity of the caecum-colon and the value of the nitrogen absorbed from the hind gut for protein synthesis in pigs. Br. J. Nutr. 46:209-219. doi:10.1079/BJN19810022

Kadim, I. T., and P. J. Moughan. 1997. Development of an ileal amino acid digestibility assay for the growing chicken—effects of time after feeding and site of sampling. Br. Poult. Sci. 38:89-95. doi:10.1080/00071669708417946

Kim, B. G., D. Y. Kil, Y. Zhang, and H. H. Stein. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles fed to pigs. J. Anim. Sci. 90:3798-3808. doi:10.2527/jas2011-4692

Köhler, T., J. Huisman, L. A. den Hartog, and R. Mosenthin. 1990. Comparison of different digesta collection methods to determine the apparent digestibilities of the nutrients at the terminal ileum in pigs. J. Sci. Food Agric. 53:465-475. doi:10.1002/jsfa.2740530405

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Kong, C., C. S. Park, J. Y. Ahn, and B. G. Kim. 2016. Relative bioavailability of $_{DL}$-methionine compared with $_{L}$-methionine fed to nursery pigs. Anim. Feed Sci. Technol. 215:181-185. doi:10.1016/j.anifeedsci.2016.03.011

Lee, J., Y. Sung, and C. Kong. 2018. Standardized ileal digestible lysine requirement of male broilers at the age of 0-10 days. Anim. Feed Sci. Technol. 241:55-62. doi:j.anifeedsci.2018.04.016

Le Floc'h, N., and B. Seve. 2007. Biological roles of tryptophan and its metabolism: Potential implications for pig feeding. Livest. Sci. 112:23-32. doi:10.1016/j.livsci.2007.07.002

Lewis, A. J. 2001. Amino acids in swine nutrition. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 131-150.

Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672-2683. doi:10.2527/1997.75102672x

Misumi, Y., and Y. Ikehara. 2013. Dipeptidyl-peptidase IV. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 3374-3379.

Möhn. S., A. M. Gillis, P. J. Moughan, and C. F. M. de Lange. 2000. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. J. Anim. Sci. 78:1510-1519. doi:10.2527/2000.7861510x

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95-117. doi:10.1016/S0377-8401(03)00163-9

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149-163. doi:10.4141/A96-044

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333-1340. doi:10.3382/ps/pey457

Ospina-Rojas, I. C., A. E. Murakami, C. R. do Amaral Duarte, P. C. Pozza, R. M. Rossi, and E. Gasparino. 2019. Performance, diameter of muscle fibers, and gene expression of mechanistic target of rapamycin in pectoralis major muscle of broilers supplemented with leucine and valine. Can. J. Anim. Sci. 99:168-178. doi:10.1139/cjas-2018-0020

Oviedo-Rondón, E. O. 2015. Model applications in poultry production and nutrition. In: N. K. Sakomura, R. M. Gous, I. Kyriazakis, and L. Hauschild, editors, Nutritional modelling for pigs and poultry. CAB International, Boston, MA. p. 125-140.

Palliyeguru, M. W. C. D., S. P. Rose, and A. M. Mackenzie. 2011. Effect of trypsin inhibitor activity in soya bean on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks. Br. Poult. Sci. 52:359-367. doi:10.1080/00071668.2011.577054

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186-192.

Parsons, C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agric. Sci. 104:469-472. doi:10.1017/S0021859600044178

Parsons, C. M. 2002. Digestibility and bioavailability of protein and amino acids. In: J. M. McNab, and K. N. Boorman, editors, Poultry feedstuffs: Supply, composition and nutritive value. CAB International, Ney York, NY. p. 115-135.

Pond, W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2005. Basic animal nutrition and feeding. 5th ed. John Wiley & Sons, Inc., Hoboken, NJ.

Ravindran, V., W. H. Hendriks, B. J. Camden, D. V. Thomas, P. C. H. Morel, and C. A. Butts. 2002. Amino acid digestibility of meat and bone meals for broiler chickens. Aust. J. Agric. Res. 53:1257-1264. doi:10.1071/AR02055

Rodrigues, I., and M. Choct. 2018. The foregut and its manipulation via feeding practices in the chicken. Poult. Sci. 97:3188-3206. doi:10.3382/ps/pey191

Sakomura, N. K., E. P. Silva, J. C. P. Dorigam, R. M. Gous, and N. St-Pierre. 2015. Modeling amino acid requirements of poultry. J. Appl. Poult. Res. 24:267-282. doi:10.3382/japr/pfv024

Sauer, W. C., A. Just, H. H. Jørgensen, M. Fekadu, and B. O. Eggum. 1980. The influence of diet composition on the apparent digestibility of crude protein and amino acids at the terminal ileum and overall in pigs. Acta Agric. Scand. 30:449-459. doi:10.1080/00015128009435293

Sauer, W. C., and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367-388. doi:10.1016/0301-6226(86)90076-X

Shen, Y. B., A. C. Weaver, and S. W. Kim. 2014. Effect of feed grade L-methionine on growth performance and gut health in nursery pigs compared with conventional DL-methionine. J. Anim. Sci. 92:5530-5539. doi:10.2527/jas2014-7830

Shirley, R. B., and C. M. Parsons. 2000. Effect of pressure processing on amino acid digestibility of meat and bone meal for poultry. Poult. Sci. 79:1775-1781. doi:10.1093/ps/79.12.1775

Spindler, H. K., R. Mosenthin, P. Rosenfelder, H. Jørgensen, K. E. Bach Knudsen, N. Sauer, J. K. Htoo, and M. Eklund. 2016. Standardized ileal digestibility of amino acids in eight genotypes of barley fed to growing pigs. Animal 10:1931-1940. doi:10.1017/S175173111600104X

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

Suryawan, A., R. A. Orellana, M. L. Fiorotto, and T. A. Davis. 2011. Triennial growth symposium: Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs. J. Anim. Sci. 89:2004-2016. doi:10.2527/jas.2010-3400

Tang, J. 2013. Pepsin A. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 27-35.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132:1-27. doi:10.1016/j.anifeedsci.2006.03.003

Trottier, N. L., and R. Manjarín. 2012. Amino acids and amino acid utilization in swine. In: L. I. Chiba, editor, Sustainable swine nutrition. John Wiley & Sons, Inc., Ames, IA. p. 81-108.

Turner, A. J. 2013. Aminopeptidase N. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 397-403.

van Leeuwen, P., D. J. van Kleef, G. J. M. van kempen, J. Huisman, and M. W. A. Verstegen. 1991. The Post Valve T-Caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. J. Anim. Physiol. Anim. Nutr. 65:183-193. doi:10.1111/j.1439-0396.1991.tb00256.x

van Milgen, J., A. Valancogne, S. Dubois, J. Y. Dourmad, B. Sève, and J. Noblet. 2008. InraPorc: A model and decision support tool for the nutrition of growing pigs. Anim. Feed Sci. Technol. 143:387-405. doi:10.1016/j.anifeedsci.2007.05.020

Voet, D., J. G. Voet, and C. W. Pratt. 2008. Fundamentals of biochemistry. 3rd ed. John Wiley & Sons, Inc., Hoboken, NJ.

Wang, J. O., M. D. Cooper, X. Iturrioz, and C. Llorens-Cortes. 2013. Glutamyl aminopeptidase. In: N. D. Rawlings, and G. Salvesen, editors, Handbook of proteolytic enzymes. 3rd ed. Academic Press, London, UK. p. 410-414.

Wang, T., and O. Adeola. 2018. 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. J. Anim. Sci. 96:2817-2825. doi:10.1093/jas/sky153

Wang, T., S. O. Osho, and O. Adeola. 2018. 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. J. Anim. Sci. 96:4731-4742. doi:10.1093/jas/sky326

Wang, T., D. Ragland, and O. Adeola. 2017. Combination of digestibility marker and fiber affect energy and nitrogen digestibility in growing pigs. Anim. Feed Sci. Technol. 230:23-29. doi:10.1016/j.anifeedsci.2017.05.012

Wang, W., J. Wang, S. Wu, X. Dong, C. Guo, H. Zhang, and G. Qi. 2019. Bioavailability of l-methionine relative to dl-methioninein broiler chickens. Ital. J. Anim. Sci. 18:1231-1238. doi:10.1080/1828051X.2019.1641433

Yen, J. T. 2001. Anatomy of the digestive system and nutritional physiology. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 31-63.

Zaboli, G. -R., G. Jalilvand, A. -A. Davarpanah, and M. Mehri. 2011. Estimation of standardized ileal digestible lysine requirement of starting broiler chicks fed soybean-and cottonseed meal-based diets. J. Anim. Vet. Adv. 10:1278-1282. doi:10.3923/javaa.2011.1278.1282

Żebrowska, T., and S. Buraczewski. 1998. Methods for determination of amino acid bioavailability in pigs. Review. Asian-Australas. J. Anim. Sci. 11:620-633. doi:10.5713/ajas.1998.620

Zhang, Y., D. Li, S. Fan, X. Piao, J. Wang, and I. K. Han. 2002. Effects of casein and protein-free diets on endogenous amino acid losses in pigs. Asian-Australas. J. Anim. Sci. 15:1634-1638. doi:10.5713/ajas.2002.1634

Table 1.1 Classification of amino acids

Item	Amino acid
Chemical structure	
Aliphatic	Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, Arg, Lys
Branched-chain	Val, Leu, Ile
Sulfur-containing	Cys, Met
Aromatic	Phe, Tyr
Heterocyclic	Trp, Pro, His
Polarity	
Nonpolar	Gly, Ala, Val, Leu, Ile, Met, Pro, Phe, Trp
Uncharged polar	Ser, Thr, Asn, Gln, Tyr, Cys
Charged polar	Lys, Arg, His, Asp, Glu
Metabolism	
Glucogenic	Thr, Arg, Met, Val, His, Cys, Glu, Gln, Asp, Asn, Gly, Ser, Pro, Ala
Ketogenic	Leu, Lys
Glucogenic and ketogenic	Ile, Phe, Tyr, Trp
Nutritional necessity	
Indispensable	His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val
Conditionally indispensable	Arg, Cys, Gln, Pro, Tyr
Dispensable	Ala, Asn, Asp, Glu, Gly, Ser

Note: adapted from D'Mello (2003), Pond et al. (2005), Voet et al. (2008), and National Research Council (2012).

Table 1.2 Ideal amino acid (AA) patterns for broiler chickens and growing-finishing pigs

	Broiler chickens	Pigs							
	Baker (2003)	van Milgen et al. (2008)[1]				National Research Council (2012)[3]			
	Age, wk	Body weight, kg				Body weight, kg			
Item	0-3	30-50	50-75	75-100	100-135	25-50	50-75	75-100	100-135
Arg	105	42.2	42.1	42.1	41.7	45.9	45.8	45.9	46.2
His	35	31.8	31.9	32.2	32.4	34.5	34.6	34.4	34.3
Ile	67	60.2	60.3	60.4	60.5	52.0	52.5	53.0	53.8
Leu	109	99.6	100.1	100.7	101.3	100.7	101.1	101.1	101.8
Lys	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Met	36	30.2	30.2	30.1	30.1	29.1	29.1	29.0	29.0
Phe	105[3]	49.5	49.9	50.6	51.1	59.5	60.3	60.1	60.9
Thr	67	64.2	64.5	65.0	65.2	60.1	62.0	63.4	65.7
Trp	16	17.9	18.0	18.3	18.4	16.9	17.3	17.5	17.8
Val	77	69.8	70.2	70.8	71.2	64.9	65.4	66.1	67.5

[1]Calculated by the requirements for standardized ileal digestible AA estimated by parameters suggested as default.
[2]Calculated by the suggested estimations for standardized ileal digestible AA requirements of mixed gender.
[3]Ratio of Phe + Tyr with Lys

Table 1.3 Mean values for the basal ileal endogenous losses of crude protein (CP) and amino acids (AA; g/kg dry matter intake) in broiler chickens and pigs fed nitrogen-free diets

	Broiler chickens	Pigs	
Item	Adeola et al. (2016)	Park et al. (2013)	Adeola et al. (2016)
CP	11.29	17.2	17.28
Indispensable AA			
Arg	0.39	0.55	0.59
His	0.18	0.18	0.17
Ile	0.37	0.34	0.30
Leu	0.56	0.57	0.50
Lys	0.39	0.42	0.40
Met	0.11	0.14	0.11
Phe	0.37	0.36	0.32
Thr	0.60	0.55	0.52
Trp	0.09	0.14	0.13
Val	0.51	0.48	0.46
Dispensable AA			
Ala	0.39	-	0.57
Asp	0.73	-	0.75
Cys	0.41	-	0.17
Glu	0.98	-	0.94
Gly	0.47	-	1.46
Pro	0.50	-	4.95
Ser	0.56	-	0.65
Tyr	0.30	-	0.35

CHAPTER 2. AMINO ACID DIGESTIBILITY OF CORN DISTILLERS' DRIED GRAINS WITH SOLUBLES WITH THE ADDITION OF CASEIN IN PIGS

A version of this chapter has been published in Journal of Animal Science.

Park, C. S., D. Ragland, and O. Adeola. 2018. Amino acid digestibility of corn distillers' dried grains with solubles with the addition of casein in pigs. J. Anim. Sci. 96:4674-4684. doi:10.1093/jas/sky309

2.1 Abstract

This experiment was conducted to determine the ileal digestibility of crude protein (CP) and amino acids (AA) in casein by regression analysis and to investigate the effects of casein in experimental diets on the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA in corn distillers' dried grains with solubles (DDGS) fed to pigs. Twenty barrows (initial body weight = 50.5 ± 4.46 kg) surgically fitted with T-cannulas at the distal ileum were used. Eighteen pigs were assigned to a triplicate 6 × 3 incomplete Latin Square design with 6 diets and 3 periods. Two pigs were used as replacements. Three diets were prepared to contain 60, 100, or 140 g/kg casein to determine the ileal digestibility of CP and AA in casein by regression analysis, and 2 diets were prepared to contain either 480 g/kg DDGS or 308 g/kg DDGS and 60 g/kg casein. Casein and DDGS were the sources of nitrogen in experimental diets. A nitrogen-free diet was prepared based on cornstarch and dextrose. Experimental periods consisted of 5 d of adaptation and 2 d of ileal digesta collection periods. The AID of CP and AA in casein linearly increased ($P < 0.01$) with increasing concentration of casein in the diets. The SID of indispensable AA in casein ranged from 94.7% (SEM = 1.16) for Ile in pigs fed the diet containing 60 g/kg casein to 103.3% (SEM = 2.21) for Arg in pigs fed the diet containing 100 g/kg casein. Except for Arg, pigs fed the diet containing DDGS and casein had greater ($P < 0.001$) SID of CP and indispensable AA than those fed the diet containing DDGS without casein. The ileal digestibility of indispensable AA in casein determined by regression analysis ranged from 96.8% (SE = 3.14) for Thr to 103.1% (SE = 4.40) for Arg. The AID and SID of CP and AA for DDGS in the diet containing casein were calculated by difference method using the ileal digestibility of CP and AA in casein determined by regression analysis. The AID of Lys for DDGS in the diet containing casein were greater ($P =$

0.035) than those without casein; however, the AID of CP and the other indispensable AA, except for Arg, Phe, and Trp, for DDGS in the diet containing casein were less ($P < 0.05$) than those without casein. The SID of Lys and Phe for DDGS in the diet containing casein were greater ($P < 0.05$) than those without casein. In conclusion, improved AA composition in semi-purified experimental diets may affect the SID of AA in low quality protein ingredients.
Key words: amino acid, casein, digestibility, distillers' dried grains with solubles, protein, swine

2.2 Introduction

Most of previous studies to determine the digestibility of crude protein (**CP**) and amino acids (**AA**) in feed ingredients have been conducted using pigs fed semi-purified diets containing feed ingredients of interest as the sole source of nitrogen (**N**). However, there may be AA deficiency in semi-purified diets because the concentration of AA in most of feed ingredients, especially for cereal grains and their byproducts, is not sufficient or balanced to maintain the normal physiological condition of pigs. Although this methodology is straightforward, impaired condition of pigs caused by feeding diets containing poor quality of AA composition may cause variation in the determined digestibility of CP and AA.

Corn distillers' dried grains with solubles (**DDGS**) has been widely used in swine diets, and many studies have been conducted to determine the standardized ileal digestibility (**SID**) of CP and AA in DDGS (Urriola et al., 2009; Kim et al., 2012; Adeola and Ragland, 2016). However, most of previous studies used semi-purified diets containing DDGS as the sole source of N, which could not provide adequate amount of AA for normal physiological function.

Casein extracted from bovine milk has been considered as a high-quality protein because of its greater concentration of digestible CP and AA (Eklund et al., 2008; Cervantes-Pahm and Stein, 2010). Therefore, the addition of casein into experimental diets containing poor quality protein can increase the quality of CP and AA in diets and reduce malnutrition of pigs. However, to the best of our knowledge, the effects of the addition of casein into semi-purified experimental diets on digestibility of CP and AA in feed ingredient have not been addressed. Therefore, the objective of this experiment was to determine the ileal digestibility of CP and AA in casein by regression analysis and to investigate the effects of addition of casein in experimental diets on the apparent ileal digestibility (**AID**) and SID of CP and AA in DDGS fed to growing pigs.

2.3 Materials and Methods

Experimental protocols with animals were reviewed and approved by the Purdue University Animal Care and Use Committee.

2.3.1 Animals, Housing, and Experimental Design

Twenty crossbred barrows with an initial body weight (**BW**) of 50.5 ± 4.46 kg were used. Pigs were surgically fitted with T-cannulas at the distal ileum described by Dilger et al. (2004) when the mean BW of pigs was approximate 20 kg and used for two digestibility trials before the current experiment. Following 2 digestion studies, pigs were fed a normal corn-soybean meal (**SBM**)-based diet for 13 d before the initiation of the current experiment. Pigs were individually housed in metabolism crates located in an environmentally controlled room. At the beginning of the experiment, 18 pigs were divided into 3 blocks based on BW and assigned to a triplicate 6 × 3 incomplete Latin Square design with 6 dietary treatments and 3 periods using a spreadsheet program (Kim and Kim, 2010). Two pigs, the heaviest and lightest among 20 pigs, were used as replacements and fed randomly assigned dietary treatments to achieve 10 observations per dietary treatment.

2.3.2 Experimental Diets, Feeding, and Sample Collection

Casein, obtained from a local supplier, and DDGS, purchased from a local ethanol plant (Iroquois Bio-energy Company, Rensselaer, IN; Table 2.1), were used as the sources of N in experimental diets. Three cornstarch-dextrose-based purified diets were formulated to contain 60, 100, or 140 g/kg casein (Table 2.2). To determine the effects of casein on the AID and SID of CP and AA in DDGS, two isonitrogenous semi-purified diets were prepared to contain either 480 g/kg DDGS or 308 g/kg DDGS and 60 g/kg casein. A N-free diet was prepared based on cornstarch and dextrose to determine the basal ileal endogenous losses of CP and AA in pigs. Diets were formulated to meet or exceed the vitamin and mineral requirement estimates of pigs with the BW ranged from 50 to 75 kg [National Research Council (**NRC**), 2012]. A 5 g/kg chromic oxide was added in all the diets as an indigestible marker.

Feeding, collection of ileal digesta, and sample processing were as previously described in Park et al. (2017).

2.3.3 Chemical Analysis

Casein, DDGS, experimental diets, and lyophilized ileal digesta samples were finely ground using a coffee grinder. Ground ingredients, diets, and ileal digesta samples were dried at 105°C for 24 h in a forced-air drying oven (Precision Scientific Co., Chicago, IL) to measure the dry matter (**DM**) concentration [method 934.01; Association of Official Analytical Chemists (**AOAC**), 2006]. Ingredients, diets, and ileal digesta samples were analyzed for N by the combustion method (TruMac® N, LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP concentration were calculated by multiplying 6.25 by the concentration of N. The analysis of AA was conducted by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Before the analysis for AA concentration, ingredients, diets, and ileal digesta samples were hydrolyzed in 6 M HCl (or BaOH for the analysis of Trp) at 110°C for 24 h under N atmosphere. For the analysis of Met and Cys, performic acid oxidation was conducted before acid hydrolysis. The concentration of AA in hydrolyzed samples were measured by high-performance liquid chromatography after post-column derivatization [AOAC, 2006; method 982.30 E (a, b, c)]. The concentration of gross energy in casein and DDGS were analyzed by an isoperibol bomb calorimeter (Model 6200, Parr Instrument Co., Moline, IL). Casein and DDGS were analyzed for ether extract [method 920.39 (A); AOAC, 2006] and ash (method 942.05; AOAC, 2006). The concentration of crude fiber (method 978.10; AOAC, 2006), neutral detergent fiber (Van Soest et al., 1991), and acid detergent fiber [method 973.18 (AD); AOAC, 2006] were analyzed by a fiber analyzer (Ankom 200 Fiber Analyzer, Ankom Technology, Macedon, NY). The concentration of Cr in experimental diets and ileal digesta samples were measured by a spectrophotometer at 450 nm (Spectronic 21D; Milton Roy Co., Rochester, NY) after the wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

2.3.4 Calculations

The AID and SID (%) of CP and AA in casein, DDGS, and diet containing DDGS and casein were calculated by the concentration of Cr and CP or AA in experimental diets and ileal digesta samples as described by Park et al. (2017). The concentration of apparent ileal digestible CP and AA [g/kg DM intake (**DMI**)] were regressed against the concentration of CP and AA (g/kg

DMI) in diets to determine the ileal digestibility (%) of CP and AA in casein using the model suggested in Akinmusire and Adeola (2009):

$$AA_D = (ID \times AA_I) - EL,$$

where AA_D represents the concentration of apparent ileal digestible CP or AA, which were calculated by multiplying AID values and the concentration of CP or AA in diets; ID represents the coefficient of ileal digestibility, which is the slope of the regression model; AA_I represents the concentration of CP or AA in diets; EL represents the endogenous losses of CP or AA as the y-intercept of the regression model.

The AID and SID of CP and AA for DDGS in the diet containing casein were calculated using the difference method described by Kong and Adeola (2014) using the following equation:

$$AID_{DG} = ID_{CAS} + [(AID_{Diet} - ID_{CAS})/P_{DG}],$$

where AID_{DG} and AID_{Diet} represent the AID of CP or AA (%) in DDGS and the diet containing DDGS and casein, respectively; ID_{CAS} represents the ileal digestibility (%) of CP or AA in casein determined by regression analysis; P_{DG} represents the proportion of CP or AA in diet contributed from DDGS, which was calculated as the concentration of CP or AA contributed from DDGS divided by the concentration of CP or AA in the diet containing DDGS and casein. The SID of CP and AA for DDGS in the diet containing casein were calculated using the same equation by replacing AID with SID.

2.3.5 Statistical Analysis

Data for the AID and SID of CP and AA in experimental diets were analyzed by MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included dietary treatment as a fixed variable and block, period, and animal within block as random variables. Two pigs for replacement was considered as another block and included in data analysis. Orthogonal polynomial contrasts were conducted to test the linear and quadratic effects of casein and to compare the AID and SID values for DDGS and diet containing DDGS and casein. The REG procedure of SAS was used for the regression analysis to estimate the ileal digestibility of CP and AA in casein. In the model, the concentration of CP or AA in the diets (g/kg DMI) was used as the independent variable, and the concentration of apparent ileal digestible CP or AA (g/kg DMI) was used as the dependent variable. The ileal digestibility (%) of CP and AA were calculated from the estimated parameters for the slopes multiplied by 100. Data for the AID and SID of CP and AA in DDGS from pigs fed diet

containing DDGS with or without the addition of casein were analyzed by two-sample, two-tailed *t* test by TTEST procedure of SAS. In all statistical analyses, experimental unit was the pig, and the significance was declared at an α level of 0.05.

2.4 Results

All pigs were in good condition during the experimental periods. Due to the limited amount of the diet containing 100 g/kg casein, one pig was excluded from the last experimental period. Therefore, there were 9 observations for the diet containing 100 g/kg of casein.

2.4.1 Chemical Analysis

The analyzed CP concentration was 880 g/kg for casein and 311 g/kg for DDGS on an as-fed basis (Table 2.1). The analyzed concentration of ether extract in DDGS was 25.2 g/kg as-fed basis. The analyzed CP and AA concentration in experimental diets were comparable with the values calculated from the concentration of CP and AA in casein and DDGS (Table 2.3).

2.4.2 Digestibility of Crude Protein and Amino Acids in Experimental Diets

The AID of CP and AA in casein linearly increased ($P < 0.01$) with increasing concentration of casein in experimental diets (Table 2.4). However, there was no quadratic effect of graded concentration of casein on the AID of CP and AA. Pigs fed the diet containing DDGS and casein had greater ($P < 0.001$) AID of CP and indispensable AA, except for Arg, compared with those fed the diet containing DDGS without casein.

The SID of CP and AA in casein were not affected by the concentration of casein in experimental diets (Table 2.5). The SID of indispensable AA in casein ranged from 94.7% for Ile in pigs fed the diet containing 60 g/kg casein to 103.3% for Arg in pigs fed the diet containing 100 g/kg casein. Similar to the AID, the SID of CP and indispensable AA, except for Arg, in the diet containing DDGS and casein were greater ($P < 0.001$) than those in the diet containing DDGS without casein.

2.4.3 Ileal Digestibility of Crude Protein and Amino Acids in Casein Determined by Regression Analysis

In the regression analyses for CP and indispensable AA, the r^2 values ranged from 0.951 for Arg to 0.996 for Met (Table 2.6). In the model for CP, the ileal digestibility was 100.8%, and the endogenous loss was 15.5 g/kg DMI with an r^2 value of 0.975. The ileal digestibility of indispensable AA ranged from 96.8% for Thr to 103.1% for Arg. The endogenous losses of indispensable AA ranged from 0.088 g/kg DMI for Met to 0.718 g/kg DMI for Lys. The ileal digestibility of dispensable AA ranged from 95.5% for Cys to 117.4% for Gly.

2.4.4 Effects of Casein on Digestibility of Crude Protein and Amino Acids in Corn Distillers' Dried Grains with Solubles

Pigs fed the DDGS diet containing casein had greater ($P = 0.035$) AID of Lys than those without casein (Table 2.7). However, the AID of CP, His, Ile, Leu, Met, Thr, and Val in DDGS without casein were greater ($P < 0.05$) than in DDGS diet containing casein. The AID of Ala, Glu, and Ser for DDGS diet containing casein were less ($P < 0.05$) than those without casein. The SID of Lys and Phe for DDGS diet containing casein were greater ($P < 0.05$) than those without casein. The SID of Asp, Gly, and Pro for DDGS diet containing casein were greater ($P < 0.05$) than those without casein.

2.5 Discussion

The analyzed concentration of CP and AA in casein agreed with the values in previous reports (Cervantes-Pahm and Stein, 2010; NRC, 2012; Brestenský et al., 2017). The analyzed concentration of CP and AA in DDGS were also within the range of values suggested in previous studies (Urriola et al., 2009; Kim et al., 2012; Kiarie et al., 2013; Xue et al., 2014), except for Arg and Lys, which were greater than previously reported values. In addition, the concentrations of GE and ether extract in DDGS were less than previously reported values. This difference may be due to the differences in the sources of corn used to produce DDGS, the ratio between distillers' dried grains to added solubles, or the time for the fermentation of starch (Spiehs et al., 2002).

The AID of CP and AA in casein linearly, but not quadratically, increased with increasing concentration of casein in experimental diets. Mariscal-Landín and Reis de Souza (2006) reported that the AID of Arg, His, Phe, Thr, Cys, and Pro linearly increased, but the AID of remaining AA,

except for Gly, quadratically increased with increasing concentration of casein in 4 experimental diets from 55.3 to 221.3 g/kg. Fan et al. (1994) reported that the AID of CP and AA in SBM quadratically increased and reached at the plateau as the concentration of CP in 6 experimental diets increases from 40 to 240 g/kg. Therefore, it may be concluded that the range of CP and AA in experimental diets used in the current experiment was not enough to observe the quadratic effects on the AID of CP and AA; however, it was appropriate to conduct the regression analysis between dietary and digestible CP and AA concentrations (Eklund et al., 2008). The SID of CP and AA in casein were similar with the values reported in Chung and Baker (1992) and Cervantes-Pahm and Stein (2010).

Regression analysis has been used to evaluate the ileal digestibility of CP and AA (Mariscal-Landín and Reis de Souza, 2006; Eklund et al., 2008; Reis de Souza et al., 2013) as well as to evaluate the digestibility of other nutrients (Johnston et al., 2013; Kim et al., 2013; Zhai and Adeola, 2013). However, the descriptive term for the digestibility (i.e., standardized or true) was not consistent although digestibility of nutrients was determined by the same regression analysis. It has been reported that the basal ileal endogenous losses of CP and AA in pigs can be estimated by regression analysis and that true ileal digestibility can be determined by correcting the total ileal endogenous losses of CP and AA, which can be determined by homoarginine method or isotope tracer dilution method, from the AID of CP and AA (Stein et al., 2007; Adeola et al., 2016). Therefore, the digestibility determined by regression analysis seems to be considered as the standardized digestibility; however, it may also be considered as the true ileal digestibility because the endogenous losses determined by regression analysis depend on feed ingredients as well as the concentration of a feed ingredient in experimental diets, which may be considered as the feed ingredient-specific endogenous losses. Therefore, to prevent the confusion with previous studies and with the various results in the current experiment, neither ‘standardized’ nor ‘true’ was used to describe the digestibility values determined by regression analysis in the current experiment. Likewise, neither ‘basal’ nor ‘total’ was used to describe the endogenous losses determined by the extrapolation to y-intercept.

Eklund et al. (2008) reported that if the range of CP and AA in experimental diets is above the plateau responses of AID values, the ileal digestibility determined by regression analysis represents the AID, and therefore, the range of CP and AA needs to be close to the origin of the coordinate. In the current experiment, although it was not clear whether the range of CP and AA

was close enough to the origin of the coordinate, the range of CP and AA was below the plateau responses of AID values. The ileal digestibility of CP and AA in casein determined by regression analysis were comparable with the values reported in previous studies using growing pigs (Jørgensen and Gabert, 2001; Mariscal-Landín and Reis de Souza, 2006). The endogenous losses of CP and AA determined by y-intercept were also comparable with the previous reports (Jørgensen and Gabert, 2001; Mariscal-Landín and Reis de Souza, 2006) except for Lys, which was greater in the current experiment. The reason for this discrepancy remains unclear; however, it may be due to the differences in the ingredient composition of experimental diets or in the sources of casein used.

Deficiency or imbalance of CP and AA in semi-purified diets has been concerned in previous studies. Liu and Adeola (2016), however, reported that the total tract digestibility of P in SBM determined by regression analysis was not affected by the addition of casein into semi-purified diets containing SBM as the sole source of P. Pedersen et al. (2007) also reported that the digestible and metabolizable energy contents in 2 sources of corn were not affected by the addition of crystalline AA into diets containing corn as the sole source of energy. In the study to determine the ileal digestibility of CP and AA, a mixture of crystalline AA was provided to pigs during the adaptation period before collecting ileal digesta samples (Pedersen et al., 2007; Strang et al., 2017). Similar approach has been used in determination of basal ileal endogenous losses of CP and AA in pigs by adding low concentration of casein into N-free diet to prevent the abnormal physiological condition caused by AA deficiency (Adeola et al., 2016).

The AID and SID of CP and AA in DDGS from pigs fed the diet containing DDGS without casein agreed with the previously reported values (Urriola et al., 2009; Kim et al., 2012; Kiarie et al., 2013; Xue et al., 2014) except for Lys, which was lower in the current experiment. It has been reported that the variation in the digestibility of Lys among sources of DDGS is greater than the other AA due to the Maillard reaction caused by heat damage (Stein and Shurson, 2009; Kim et al., 2012; Almeida et al., 2013). Therefore, it may be concluded that DDGS used in the current experiment may be treated in a high temperature during the process, and thus bioavailability of Lys decreased.

Digestibility of CP and AA in pigs may increase from an upsurge in digestive enzyme secretion or increased expression of peptide or AA transporters in the small intestine or both. Morales et al. (2017) reported that the activity of trypsin and chymotrypsin increased in the

duodenum and jejunum of pigs fed the diet containing 281 g/kg CP compared with pigs fed the diet containing 192 g/kg CP with the supplementation of crystalline AA. However, in the current experiment, two diets containing DDGS with or without casein were formulated to contain the same concentration of CP. Moreover, Valette et al. (1992) reported that the activity of chymotrypsin in pigs fed the diet containing casein was less than that in pigs fed the diet containing rapeseed-concentrate protein, although the diets were prepared to contain the same concentration of CP as 120 g/kg from casein or rapeseed-concentrate protein as the sole source of N. Therefore, it may be concluded that the pancreatic enzyme activity in pigs used in the current experiment was not affected by the diets. Nevertheless, the SID of Lys and Phe increased when casein was added in the experimental diet. Therefore, it may be speculated that the expression or activity of transporters of peptide or AA in the small intestine increased due to the increased protein quality of the diet containing DDGS and casein. However, the evidence to explain the increased expression or activity of peptide or AA transporters is limited. Gilbert et al. (2008) reported that the mRNA expression of transporters in broiler chickens were affected by the quality of protein sources in diets with the results showing that the mRNA expression of intestinal peptide transporter 1 (**PepT1**) and Na^+-independent cationic and zwitterionic amino acid transporter (**$b^{0,+}$AT**) in the small intestine of broiler chickens fed the diet containing 467 g/kg SBM were greater than those fed the diet containing 342 g/kg corn gluten meal. Because Lys is one of the main substrates for $b^{0,+}$AT (Bröer, 2008), it may partially explain the increased digestibility of Lys in the current experiment. However, because the substrates for PepT1 are di- and tripeptide (Herrera-Ruiz and Knipp, 2003), it remains unclear why the digestibility of Phe, but not all AA, increased. Further research is needed to verify the relationship between dietary protein quality and the expression or activity of peptide or AA transporters.

The standardized ileal digestible AA concentration in all experimental diets in the current study except for the diet containing 140 g/kg casein were less than suggested standardized ileal digestible AA requirement in NRC (2012). However, the SID of AA in diets containing 60 or 100 g/kg casein were not underestimated based on the results that there were no linear or quadratic effects of graded concentration of casein on the SID of AA in casein. Thus, the digestibility of AA from readily digestible protein was not affected by deficiency of AA.

In the current experiment, two diets containing DDGS with or without casein were formulated to contain the same concentration of CP to prevent the potential effect of dietary CP

concentration on digestibility of CP and AA. However, when the digestibility of CP and AA in DDGS were calculated from pigs fed the diet containing DDGS and casein, the CP and AA contributed from casein were deducted by their digestibility values, which eventually decreased the concentration of CP and AA contributed from DDGS in the diet. Due to the decreased CP contributed from DDGS, the AID of CP and several AA for DDGS in the diet containing casein decreased compared with those without casein. This result was consistent with previous reports which suggested that the AID of CP and AA increased with increasing concentration of dietary CP concentration (Fan et al., 1994) due to the decreased proportional contribution of endogenous losses of CP and AA in ileal digesta (Kong and Adeola, 2014). However, it remains unclear whether increased SID of Lys and Phe for DDGS in the diet containing casein was due to the addition of casein or decreased contribution of CP from DDGS. Although Adebiyi et al. (2015) reported that the dietary concentration of CP as 150 or 190 g/kg did not affect the AID and SID of CP and AA in pigs fed the diets containing corn and DDGS, further research is needed to clarify whether the supplementation of casein increases the digestibility of AA in DDGS regardless of dietary CP concentration.

In conclusion, the ileal digestibility of CP and AA determined by regression analysis were close to 100%, which indicated that the CP and AA in casein were highly digestible in the small intestine of pigs. The addition of casein in DDGS diet increased the SID of Lys and Phe compared with values for DDGS diet without casein. Therefore, improved AA composition in the semi-purified experimental diet may increase the SID of several AA in test ingredients with low quality of AA composition.

2.6 Literature Cited

Adebiyi, A. O., D. Ragland, O. Adeola, and O. A. Olukosi. 2015. Apparent or standardized ileal digestibility of amino acids of diets containing different protein feedstuffs fed at two crude protein levels for growing pigs. Asian-Australas. J. Anim. Sci. 28:1327-1334. doi:10.5713/ajas.14.0914

Adeola, O., and D. Ragland. 2016. Comparative ileal amino acid digestibility of distillers' grains for growing pigs. Anim. Nutr. 2:262-266. doi:10.1016/j.aninu.2016.07.008

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274-283. doi:10.1016/j.anifeedsci.2016.06.004

Akinmusire, A. S., and O. Adeola. 2009. True digestibility of phosphorus in canola and soybean meals for growing pigs: Influence of microbial phytase. J. Anim. Sci. 87:977-983. doi: 10.2527/jas.2007-0778

Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. J. Anim. Sci. Biotechnol. 4:44. doi:10.1186/2049-1891-4-44

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Brestenský, M., S. Nitrayová, and P. Patráš. 2017. Ileal endogenous losses in pigs feeding a protein-free diet or diets with different contents of casein or crystalline amino acids. Arch. Anim. Nutr. 71:210-218. doi:10.1080/1745039X.2017.1312832

Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. Physiol. Rev. 88:249-286. doi:10.1152/physrev.00018.2006

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674-2683. doi:10.2527/jas.2009-2677

Chung, T. K., and D. H. Baker. 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: Comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70:3781-3790. doi:10.2527/1992.70123781x

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

Eklund, M., R. Mosenthin, H. P. Piepho, and M. Rademacher. 2008. Estimates of basal ileal endogenous losses of amino acids by regression analysis and determination of standardized ileal amino acid digestibilities from casein in newly weaned pigs. J. Sci. Food Agric. 88:641-651. doi:10.1002/jsfa.3129

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: Effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859. doi:10.2527/1994.72112851x

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634. doi:10.4141/cjas79-081

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb Jr., and E. A. Wong. 2008. Dietary protein composition influences abundance of peptide and amino acid transporter messenger ribonucleic acid in the small intestine of 2 lines of broiler chicks. Poult. Sci. 89:1663-1676. doi:10.3382/ps.2010-00801

Herrera-Ruiz, D., and G. T. Knipp. 2003. Current perspectives on established and putative mammalian oligopeptide transporters. J. Pharm. Sci. 92:691-714. doi:10.1002/jps.10303

Johnston, A. M., Woyengo, T. A., and C. M. Nyachoti. 2013. True digestive utilization of phosphorus in pea (*Pisum sativum*) fed to growing pigs. Anim. Feed Sci. Technol. 185:169-174. doi:10.1016/j.anifeedsci.2013.07.010

Jørgensen, H., and V. M. Gabert. 2001. The ileal digestion of semipurified diets containing increasing levels of casein and the use of the regression method to estimate the endogenous flow of amino acids at the distal ileum of growing pigs. Proceedings of the 8th Symposium of Digestive Physiology of Pigs, Uppsala, Sweden. p. 198-201. doi:10.1079/9780851995175.0198

Kiarie, E., P. Lopez, C. Furedi, and C. M. Nyachoti. 2013. Amino acids and energy utilization in zero-tannin faba bean and co-fermented wheat and corn dried distillers grains with solubles fed to growing pigs. J. Anim. Sci. 91:1728-1735. doi:10.2527/jas2012-5485

Kim, B. G., D. Y. Kil, and H. H. Stein. 2013. In growing pigs, the true ileal and total tract digestibility of acid hydrolyzed ether extract in extracted corn oil is greater than in intact sources of corn oil or soybean oil. J. Anim. Sci. 91:755-763. doi:10.2527/jas2011-4777

Kim, B. G., D. Y. Kil, Y. Zhang, and H. H. Stein. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles fed to pigs. J. Anim. Sci. 90:3798-3808. doi:10.2527/jas2011-4692

Kim. B. G., and T. Kim. 2010. A program for making completely balanced Latin Square designs employing a systemic method. Rev. Colomb. Cienc. Pecu. 23:277-282.

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Liu, J. B., and O. Adeola. 2016. Casein supplementation does not affect the estimates of true total tract digestibility of phosphorus in soybean meal for growing pigs determined by the regression method. Asian-Australas. J. Anim. Sci. 29:1625-1631. doi:10.5713/ajas.15.0825

Mariscal-Landín, G., and T. C. Reis de Souza. 2006. Endogenous ileal losses of nitrogen and amino acids in pigs and piglets fed graded levels of casein. Arch. Anim. Nutr. 60:454-466. doi:10.1080/17450390600973642

Morales, A., L. Buenabad, G. Castillo, L. Vázquez, S. Espinoza, J. K. Htoo, and M. Cervantes. 2017. Dietary levels of protein and free amino acids affect pancreatic proteases activities, amino acids transporters expression and serum amino acid concentrations in starter pigs. J. Anim. Physiol. Anim. Nutr. 101:723-732. doi:10.1111/jpn.12515

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. National Academic Press, Washington, DC.

Pedersen, C., M. G. Boersma, and H. H. Stein. 2007. Energy and nutrient digestibility in NutriDense corn and other cereal grains fed to growing pigs. J. Anim. Sci. 85:2473-2483. doi:10.2527/jas.2006-620

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110-3119. doi: 10.2527/jas2017.1404

Reis de Souza, T. C., A. A. Barreyro., and G. Mariscal-Landín. 2013. Estimation of endogenous protein and amino acid ileal losses in weaned piglets by regression analysis using diets with graded levels of casein. J. Anim. Sci. Biotechnol. 4:36. doi:10.1186/2049-1891-4-36

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645. doi:10.2527/2002.80102639x

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742

Stein, H. H., and G. C. Shurson. 2009. Board-invited review: The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci. 87:1292-1303. doi:10.2527/jas.2008-1290

Strang, E. J. P., M. Eklund, P. Rosenfelder, J. K. Htoo, and R. Mosenthin. 2017. Variations in the chemical composition and standardized ileal digestibility of amino acids in eight genotypes of triticale fed to growing pigs. J. Anim. Sci. 95:1614-1625. doi:10.2527/jas2016.1238

Urriola, P. E., D. Hoehler, C. Pedersen, H. H. Stein, and G. C. Shurson. 2009. Amino acid digestibility of distillers dried grains with solubles, produced from sorghum, a sorghum-corn blend, and corn fed to growing pigs. J. Anim. Sci. 87:2574-2580. doi:10.2527/jas.2008-1436

Valette. P., H. Malouin, T. Corring, L. Savoie, A. M. Gueugneau, and S. Berot. 1992. Effects of diets containing casein and rapeseed on enzyme secretion from the exocrine pancreas in the pig. Br. J. Nutr. 67:215-222. doi:10.1079/BJN19920025

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Xue, P. C., D. Ragland, and O. Adeola. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937-3944. doi:10.2527/jas.2014-7815

Zhai. H., and O. Adeola. 2013. True total-tract digestibility of phosphorus in corn and soybean meal for fifteen-kilogram pigs are additive in corn-soybean meal diet. J. Anim. Sci. 91:219-224. doi:10.2527/jas2012-5295

Table 2.1 Analyzed chemical composition of casein and corn distillers' dried grains with solubles (DDGS), g/kg as-fed basis

	Ingredient	
Item	Casein	DDGS
Dry matter	923	874
Gross energy, kcal/kg	5,419	4,089
Crude protein (nitrogen × 6.25)	880	311
Ether extract	3.6	25.2
Crude fiber	-	35.6
Ash	12.6	39.6
Neutral detergent fiber	-	122
Acid detergent fiber	-	74.5
Indispensable amino acid		
Arg	26.9	19.7
His	25.3	8.1
Ile	50.2	14.3
Leu	87.4	28.2
Lys	69.2	16.2
Met	27.7	5.0
Phe	46.1	16.0
Thr	39.2	11.8
Trp	14.0	3.7
Val	60.4	15.4
Dispensable amino acid		
Ala	26.1	16.0
Asp	60.3	30.4
Cys	3.3	5.0
Glu	180.9	52.3
Gly	15.9	13.3
Pro	107.0	18.4
Ser	40.0	13.1
Tyr	50.1	11.2

Note: approximately 1-kg subsamples were collected for analyses.
Note: mean values of duplicate analyses except for ether extract (n = 6) in DDGS and amino acids (n = 1) in casein.

Table 2.2 Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[1]					
Casein, g/kg:	60	100	140	-	60	
Item DDGS, g/kg:	-	-	-	480	308	NFD
Cornstarch	710.1	670.1	630.1	340.6	452.6	768.6
Casein	60.0	100.0	140.0	0.0	60.0	0.0
DDGS	0.0	0.0	0.0	480.0	308.0	0.0
Dextrose	100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil	30.0	30.0	30.0	30.0	30.0	30.0
Cellulose[2]	40.0	40.0	40.0	0.0	0.0	40.0
Limestone	10.5	10.5	10.5	13.5	13.5	9.5
Monocalcium phosphate	12.5	12.5	12.5	4.0	4.0	15.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0
Potassium carbonate	4.0	4.0	4.0	0.0	0.0	4.0
Magnesium oxide	1.0	1.0	1.0	0.0	0.0	1.0
Vitamin premix[3]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[4]	0.9	0.9	0.9	0.9	0.9	0.9
Selenium premix[5]	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide premix[6]	25.0	25.0	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000	1,000	1,000

[1]DDGS = corn distillers' dried grains with solubles; NFD = nitrogen-free diet.
[2]Solka-Floc® 40 FCC, International Fiber Corporation, Urbana, OH.
[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 of complete diet.

Table 2.3 Analyzed concentration of crude protein (CP) and amino acids (AA) in experimental diets, g/kg as-fed basis

	Diet[1]					
Casein, g/kg:	60	100	140	-	60	
Item DDGS, g/kg:	-	-	-	480	308	NFD
CP (nitrogen × 6.25)	55.9	86.8	123.5	133.0	136.5	7.52
Indispensable AA						
Arg	1.60	2.30	3.30	5.40	5.00	0.10
His	1.60	2.40	3.50	3.40	3.60	0.00
Ile	3.40	5.10	7.60	5.35	6.65	0.10
Leu	5.80	8.70	12.40	14.80	14.85	0.20
Lys	4.60	6.80	9.80	3.95	6.65	0.10
Met	1.70	2.50	3.60	2.35	3.15	0.10
Phe	3.10	4.50	6.40	6.25	6.85	0.10
Thr	2.60	3.90	5.50	4.90	5.55	0.10
Trp	0.80	1.20	1.60	1.00	1.25	0.00
Val	4.00	5.90	8.50	6.55	7.80	0.20
Dispensable AA						
Ala	1.80	2.70	3.80	9.10	7.55	0.20
Asp	4.00	6.00	8.50	8.70	9.30	0.20
Cys	0.20	0.30	0.50	2.50	1.90	0.10
Glu	12.70	19.10	26.90	19.80	24.10	0.70
Gly	1.10	1.60	2.30	5.55	4.60	0.10
Pro	6.70	10.30	14.80	9.85	12.50	0.10
Ser	2.90	4.30	6.00	5.35	5.95	0.10
Tyr	1.70	2.40	3.30	3.85	4.80	0.10

[1]DDGS = corn distillers' dried grains with solubles; NFD = nitrogen-free diet.

Table 2.4 Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in experimental diets fed to pigs

		Diet[1]						*P*-value[2]	
	Casein, g/kg:	60	100	140	-	60			
Item	DDGS, g/kg:	-	-	-	480	308	SEM	Linear	Quadratic	D vs. D + C
CP		75.2	85.5	89.1	64.3	74.9	1.83	< 0.001	0.155	< 0.001
Indispensable AA										
Arg		71.5	82.5	87.9	75.9	80.0	2.21	< 0.001	0.314	0.179
His		88.4	93.3	94.5	72.7	80.5	1.08	< 0.001	0.179	< 0.001
Ile		86.6	92.3	93.7	71.9	81.3	1.16	< 0.001	0.099	< 0.001
Leu		90.6	93.8	95.1	82.1	87.0	0.92	< 0.001	0.364	< 0.001
Lys		85.4	90.6	92.6	33.6	68.4	1.75	0.002	0.439	< 0.001
Met		94.3	96.6	96.8	76.3	86.9	0.70	0.002	0.137	< 0.001
Phe		89.9	93.1	94.5	75.0	83.6	1.08	0.002	0.452	< 0.001
Thr		77.9	84.4	87.7	56.6	69.7	1.74	< 0.001	0.472	< 0.001
Trp		84.9	90.5	91.5	68.3	80.5	1.68	0.006	0.282	< 0.001
Val		85.2	90.5	92.2	66.7	77.4	1.16	< 0.001	0.197	< 0.001
Dispensable AA										
Ala		69.6	81.0	85.6	75.3	78.1	2.45	< 0.001	0.256	0.399
Asp		81.3	88.7	90.3	59.3	71.5	1.82	< 0.001	0.193	< 0.001
Cys		24.7	47.8	67.0	63.7	65.9	6.44	< 0.001	0.803	0.802
Glu		89.6	93.9	94.9	77.7	85.3	0.89	< 0.001	0.141	< 0.001
Gly		6.0	52.0	65.0	55.1	60.5	8.57	< 0.001	0.106	0.627
Pro		53.9	77.4	84.1	63.3	79.9	4.94	< 0.001	0.135	0.009
Ser		78.6	85.7	89.1	65.9	73.6	1.33	< 0.001	0.286	< 0.001
Tyr		86.8	90.9	92.4	70.7	83.3	1.42	0.003	0.436	< 0.001

Note: each least squares mean represents 10 observations except for the diet containing 100 g/kg casein (9 observations).

[1]DDGS = corn distillers' dried grains with solubles.

[2]Linear = linear effect of casein; Quadratic = quadratic effect of casein; D vs. D + C = contrast between diet containing DDGS and that containing DDGS plus casein.

Table 2.5 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in experimental diets fed to pigs

		Diet[1]							
	Casein, g/kg:	60	100	140	-	60		*P*-value[2]	
Item	DDGS, g/kg:	-	-	-	480	308	SEM	Linear	Quadratic	D vs. D + C
CP		98.0	100.2	99.4	73.8	84.2	1.83	0.591	0.518	< 0.001
Indispensable AA										
Arg		101.3	103.3	102.3	84.6	89.9	2.21	0.737	0.598	0.085
His		97.7	99.5	98.7	76.8	84.7	1.08	0.479	0.345	< 0.001
Ile		94.7	97.8	97.3	76.7	85.4	1.16	0.072	0.184	< 0.001
Leu		98.1	98.8	98.6	84.9	89.9	0.92	0.667	0.637	< 0.001
Lys		96.6	98.1	97.8	46.2	76.3	1.75	0.574	0.639	< 0.001
Met		98.6	99.6	98.9	79.4	89.2	0.70	0.711	0.239	< 0.001
Phe		98.8	99.3	98.8	79.3	87.7	1.08	0.973	0.691	< 0.001
Thr		96.4	96.7	96.4	66.3	78.5	1.74	0.989	0.879	< 0.001
Trp		95.8	97.8	96.9	76.9	87.2	1.68	0.616	0.505	< 0.001
Val		96.8	98.4	97.6	73.5	83.4	1.16	0.562	0.397	< 0.001
Dispensable AA										
Ala		97.3	99.5	98.7	80.6	84.7	2.45	0.678	0.611	0.213
Asp		97.2	99.3	97.7	66.5	78.5	1.82	0.815	0.400	< 0.001
Cys		92.0	92.8	93.8	69.3	73.4	6.44	0.832	0.990	0.638
Glu		95.6	97.9	97.7	81.5	88.6	0.89	0.076	0.269	< 0.001
Gly		104.5	120.0	112.1	73.8	84.1	8.57	0.503	0.249	0.365
Pro		107.4	112.3	108.3	97.7	108.3	4.94	0.890	0.421	0.086
Ser		92.9	95.3	96.0	73.4	80.7	1.33	0.091	0.601	< 0.001
Tyr		98.3	99.1	98.3	76.2	87.8	1.42	0.996	0.647	< 0.001

Note: each least squares mean represents 10 observations except for the diet containing 100 g/kg casein (9 observations).

Note: values for the standardized ileal digestibility was calculated by correcting basal ileal endogenous losses of CP and AA (g/kg dry matter intake) in pigs fed nitrogen-free diet from values for the apparent ileal digestibility: CP, 14.1; Arg, 0.529; His, 0.165; Ile, 0.306; Leu, 0.481; Lys, 0.568; Met, 0.082; Phe, 0.308; Thr, 0.533; Trp, 0.096; Val, 0.514; Ala, 0.553; Asp, 0.704; Cys, 0.150; Glu, 0.846; Gly, 1.204; Pro, 3.984; Ser, 0.460; Tyr, 0.218.

[1]DDGS = corn distillers' dried grains with solubles.

[2]Linear = linear effect of casein; Quadratic = quadratic effect of casein; D vs. D + C = contrast between diet containing DDGS and that containing DDGS plus casein.

Table 2.6 Ileal digestibility (%) and endogenous losses [g/kg dry matter intake (DMI)] of crude protein (CP) and amino acids (AA) in pigs fed diets containing graded concentration of casein determined by regression analysis

	Regression analysis parameters[1]						
	Slope		Intercept		Statistical parameters		
Item	Digestibility	SE	Endogenous loss	SE	RMSE	r^2	*P*-value
CP	100.8	3.07	15.5	3.18	5.197	0.975	< 0.001
Indispensable AA							
Arg	103.1	4.40	0.548	0.1226	0.188	0.951	< 0.001
His	99.9	1.77	0.196	0.0516	0.084	0.991	< 0.001
Ile	99.5	1.79	0.464	0.1125	0.189	0.991	< 0.001
Leu	99.1	1.61	0.539	0.1682	0.266	0.993	< 0.001
Lys	99.7	2.06	0.718	0.1693	0.268	0.988	< 0.001
Met	99.2	1.13	0.088	0.0343	0.054	0.996	< 0.001
Phe	98.9	1.89	0.304	0.1021	0.156	0.990	< 0.001
Thr	96.8	3.14	0.546	0.1460	0.228	0.971	< 0.001
Trp	98.6	3.17	0.117	0.0439	0.063	0.972	< 0.001
Val	98.9	2.08	0.589	0.1482	0.234	0.988	< 0.001
Dispensable AA							
Ala	99.7	4.82	0.590	0.1550	0.241	0.938	< 0.001
Asp	98.6	3.17	0.737	0.2272	0.357	0.972	< 0.001
Cys	95.5	9.10	0.158	0.0362	0.069	0.796	< 0.001
Glu	99.7	1.51	1.369	0.3435	0.536	0.994	< 0.001
Gly	117.4	15.04	1.293	0.2912	0.453	0.681	< 0.001
Pro	112.4	7.70	4.265	0.9526	1.559	0.884	< 0.001
Ser	99.3	2.59	0.661	0.1320	0.201	0.981	< 0.001
Tyr	98.5	2.76	0.218	0.0784	0.111	0.979	< 0.001

Note: regression analysis was conducted with the concentration of CP or AA (g/kg DMI) and the concentration of apparent ileal digestible CP or AA (g/kg DMI) in diets containing 60, 100, and 140 g/kg casein (29 observations).
[1]Ileal digestibility was estimated by multiplying the slope by 100, and the endogenous loss was estimated by the absolute value of y-intercept.

Table 2.7 Apparent and standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in corn distillers' dried grains with solubles (DDGS) with or without the addition of casein fed to pigs

	Apparent ileal digestibility				Standardized ileal digestibility			
	DDGS				DDGS			
Item	– Casein	+ Casein	SE	*P*-value	– Casein	+ Casein	SE	*P*-value
CP	64.3	61.2	1.28	0.024	73.8	75.6	1.28	0.171
Indispensable AA								
Arg	75.9	74.5	1.21	0.278	84.6	87.1	1.21	0.050
His	72.8	68.9	1.18	0.004	76.9	75.5	1.18	0.267
Ile	71.9	69.0	1.21	0.026	76.8	76.0	1.21	0.516
Leu	82.1	79.7	0.76	0.005	84.9	84.4	0.76	0.520
Lys	33.6	41.6	3.53	0.035	46.2	56.5	3.53	0.009
Met	76.4	73.6	0.78	0.002	79.4	78.4	0.78	0.222
Phe	75.0	75.2	1.03	0.853	79.3	81.6	1.03	0.044
Thr	56.6	51.7	1.88	0.019	66.3	66.5	1.88	0.884
Trp	68.4	67.6	2.06	0.722	76.9	79.2	2.06	0.287
Val	66.7	61.4	1.45	0.002	73.4	72.0	1.45	0.332
Dispensable AA								
Ala	75.3	71.0	0.92	< 0.001	80.6	79.8	0.92	0.403
Asp	59.4	61.3	1.84	0.328	66.6	70.9	1.84	0.030
Cys	63.8	61.9	1.64	0.259	69.3	70.4	1.64	0.524
Glu	77.8	75.5	0.89	0.022	81.5	81.0	0.89	0.583
Gly	55.1	51.3	2.53	0.157	73.8	80.3	2.53	0.019
Pro	62.9	56.8	4.68	0.212	97.3	117.1	4.68	< 0.001
Ser	65.8	58.0	1.83	< 0.001	73.3	69.5	1.83	0.050
Tyr	70.8	69.9	1.40	0.547	76.3	78.5	1.40	0.127

Note: each mean represents 10 observations.

Note: digestibility of CP and AA in the diet containing DDGS with the addition of casein at 60 g/kg were calculated using the difference method with the ileal digestibility of CP and AA in casein determined by regression analysis.

CHAPTER 3. AMINO ACID DIGESTIBILITY IN CORN DISTILLERS' DRIED GRAINS WITH SOLUBLES FOR GROWING PIGS WITH GRADED CONCENTRATION OF CASEIN AT TWO DIETARY CRUDE PROTEIN CONCENTRATIONS

3.1 Abstract

Determination of amino acid (AA) digestibility in corn distillers' dried grains with solubles (DDGS) may be affected by characteristics of experimental diets such as protein quality of diets or the concentration of crude protein (CP) contributed from DDGS. Studies were conducted to determine the effects of graded concentration of casein in experimental diets on standardized ileal digestibility (SID) of AA in DDGS (Exp. 1) and the SID of AA in DDGS responses to DDGS concentration in and casein addition to experimental diets (Exp. 2). In Exp. 1, 20 pigs (initial body weight = 45.3 ± 1.80 kg), surgically fitted with T-cannulas at the distal ileum were allocated to a quadruplicate 5 × 2 incomplete Latin square design with 5 diets and 2 periods. Experimental diets consisted of 4 isonitrogenous diets containing graded concentration of casein with decreasing concentration of DDGS and a nitrogen-free diet. Each experimental period was composed of 5-d adaptation and 2-d ileal digesta collection periods. The SID of AA in experimental diets linearly increased ($P < 0.001$) as dietary casein concentrations increase. However, the SID of indispensable AA, except for Arg and Lys, in DDGS linearly decreased ($P < 0.05$) with increasing concentration of casein in experimental diets. In Exp. 2, the same 20 pigs (initial body weight = 52.8 ± 2.99 kg) and experimental design as Exp. 1 were used with different dietary treatments. Experimental diets were prepared as a 2 × 2 factorial arrangement with the concentration of DDGS at 466.8 or 155.6 g/kg and the addition of casein at 0 or 110 g/kg in experimental diets, plus and a nitrogen-free diet. Experimental procedures were the same as Exp. 1. Pigs fed the diet containing 466.8 g/kg DDGS without casein had greater ($P < 0.05$) SID of Arg, Ile, Lys, Met, Thr, and Val in diets than those fed the diet containing 155.6 g/kg DDGS without casein but had less ($P < 0.05$) SID values than those fed the diets containing DDGS and casein. Pigs fed experimental diets containing 466.8 g/kg DDGS had greater ($P < 0.01$) SID of indispensable AA, except for Trp, in DDGS than those fed diets containing 155.6 g/kg DDGS regardless of the addition of casein. The addition of casein in experimental diets did not affect the SID of CP and indispensable AA, except for Arg, in DDGS. In conclusion, the addition of casein did not affect the SID of most AA in DDGS, but the low

concentration of CP contributed from DDGS in experimental diets reduced the SID of most AA in DDGS.

Key words: amino acid, casein, corn distillers' dried grains with solubles, digestibility, protein, swine

3.2 Introduction

The standardized ileal digestibility (**SID**) of amino acids (**AA**) in feed ingredients has been generally determined by the direct method in which feed ingredients of interest are the sole source of nitrogen (**N**) in experimental diets (Kong and Adeola, 2014). However, if feed ingredients of interest contain limited or imbalanced concentration of AA to support the normal physiological condition of pigs, feeding experimental diets may cause a deficiency of AA which can negatively affect the ability of pigs to digest and absorb AA. Therefore, determination of the SID of AA in feed ingredients may be affected by protein quality of experimental diets.

Deficiency of AA in pigs has been discussed in the estimation of the basal ileal endogenous losses (**BEL**) of AA in pigs by feeding a N-free diet (**NFD**; Adeola et al., 2016). One of the methods to mitigate the AA deficiency is feeding a diet containing low concentration of casein, which contains high concentration of digestible AA (Adeola et al., 2016). Therefore, the addition of casein in experimental diets for AA digestibility may also minimize the potential negative effects of AA deficiency on determination of the SID of AA.

Park et al. (2018) reported that the addition of 60 g/kg casein in semi-purified experimental diets prepared to determine the SID of AA in corn distillers' dried grains with solubles (**DDGS**) increased the SID of Lys and Phe in DDGS. These findings indicated that the improved AA composition in experimental diets by the addition of casein may increase the SID of AA in DDGS, which contains insufficient and imbalanced AA to support the normal physiological function of pigs. Therefore, it was further hypothesized in the current study that the increasing concentration of casein in experimental diets will not affect the SID of AA in DDGS. In addition, in the previous study of Park et al. (2018), the concentration of DDGS in the experimental diet was reduced when casein was added to provide the same concentration of crude protein (**CP**) as the diet containing DDGS without casein. Consequently, it was unclear whether improvements in the SID of Lys and Phe in DDGS were due to the addition of casein or due to the decreased concentration of CP in the experimental diet contributed from DDGS. Therefore, the second hypothesis of the current study

was that the concentration of CP in diets contributed from DDGS and the addition of casein in diets will not affect the SID of AA in DDGS. To address the aforementioned hypotheses, 2 experiments were conducted to determine the effects of graded concentration of casein in experimental diets on SID of AA in DDGS (Exp. 1) and to determine the effects of DDGS concentration and casein addition in experimental diets on SID of AA in DDGS (Exp. 2).

3.3 Materials and Methods

Protocols for animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

3.3.1 Experiment 1: Amino Acid Digestibility Responses to Graded Concentration of Casein in Experimental Diets

Twenty barrows with an initial body weight (**BW**) of 45.3 ± 1.80 kg were surgically fitted with T-cannulas at the distal ileum as described by Dilger et al. (2004). Pigs were divided into 4 blocks based on BW, and then allocated to a quadruplicate 5 × 2 incomplete Latin Square design with 5 dietary treatments and 2 periods. Pigs were individually housed in metabolism crates equipped with a feeder and a nipple drinker during the experimental periods.

Two isonitrogenous diets were prepared to contain 466.8 g/kg DDGS or 165 g/kg casein as the sole source of N based on cornstarch and dextrose (Table 3.1). Purified cellulose was added into the diet containing casein at 40 g/kg to provide dietary fiber. Both diets were prepared to meet or exceed the vitamin and mineral requirements for pigs [National Research Council (**NRC**), 2012]. Thereafter, those two diets were blended to prepare 2 additional isonitrogenous diets containing 55 or 110 g/kg casein with decreasing concentration of DDGS at 311.2 or 155.6 g/kg. The NFD was also prepared to estimate the BEL of CP and AA in pigs. Chromic oxide was added in all diets at 5 g/kg as an indigestible marker.

Feeding, collection of ileal digesta, and processing of collected ileal digesta samples were conducted as described in Park et al. (2017).

3.3.2 Experiment 2: Amino Acid Digestibility Responses to Dietary Concentration of Corn Distillers' Dried Grains with Solubles with or without Casein

Twenty barrows used in Exp. 1 were also used in this experiment. After the termination of Exp. 1, pigs were fed a normal corn-soybean meal-based standard grower diet (194 g CP/kg) for 7 d before the initiation of Exp. 2. Pigs (initial BW = 52.8 ± 2.99 kg) were allocated to a quadruplicate 5 × 2 incomplete Latin square design with 5 dietary treatments and 2 periods. The procedure for allotment of pigs and housing environment were the same as in Exp. 1.

Three of the experimental diets used in Exp. 1 were also used in this experiment, which includes the diet containing 466.8 g/kg DDGS without casein, the diet containing 155.6 g/kg DDGS and 110 g/kg casein, and the NFD. Two additional diets were prepared to contain 466.8 g/kg DDGS and 110 g/kg casein or 155.6 g/kg DDGS without casein (Table 3.1). Therefore, dietary treatments were prepared as a 2 × 2 factorial arrangement with the concentration of DDGS at 466.8 or 155.6 g/kg, which contributed the concentration of CP at 130 or 44 g/kg, respectively, and the addition of casein at 0 or 110 g/kg in experimental diets.

Feeding, collection of ileal digesta, and processing of collected ileal digesta samples were conducted as described in Park et al. (2017).

3.3.3 Chemical Analysis

Representative samples of DDGS, casein, experimental diets, and lyophilized ileal digesta were finely ground (< 0.75 mm) by a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany) before chemical analysis. Ground samples were analyzed for dry matter by drying at 105°C for 24 h in a forced-air drying oven [Precision Scientific Co., Chicago, IL; method 934.01; Association of Official Analytical Chemists (**AOAC**), 2006] and for N by combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000). The concentration of CP was calculated by multiplying the concentration of N by 6.25. The concentration of AA in samples were analyzed by high-performance liquid chromatography after post-column derivatization [method 982.30 E (a, b, c); AOAC, 2006] after the hydrolysis of samples in 6 M HCl (or BaOH for the analysis of Trp) at 110°C for 24 h under N atmosphere. Samples were oxidized by performic acid before the acid hydrolysis for the analysis of Met and Cys. The analysis of AA was conducted at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Samples of DDGS and casein were analyzed for gross energy using an isoperibol bomb calorimeter

(model 6200; Parr Instrument Co., Moline, IL), ether extract [method 920.39 (A); AOAC, 2006], and ash (method 942.05; AOAC, 2006). Neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) concentrations in DDGS were analyzed using a fiber analyzer (Ankom 2000 Fiber Analyzer; Ankom Technology, Macedon, NY) according to Van Soest et al. (1991) for NDF and AOAC [method 973.18 (AD); 2006] for ADF. The concentration of Cr in samples of experimental diets and ileal digesta was analyzed by spectrophotometry (Spark 10M; Tecan Group Ltd., Männedorf, Switzerland) at 450 nm of absorption after the wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

3.3.4 Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and SID of CP and AA in experimental diets were calculated by the index method using the concentration of Cr, CP, and AA in experimental diets and ileal digesta samples (Park et al., 2017). The BEL of CP and AA in pigs were estimated from pigs fed NFD as described in Park et al. (2017). The SID of CP and AA in DDGS from pigs fed diets containing both DDGS and casein were calculated by the difference method described in Park et al. (2018) with the SID of CP and AA in casein determined by pigs fed the diet containing casein without DDGS in Exp. 1.

Data for the SID of CP and AA in experimental diets and DDGS were analyzed using the mixed linear model procedure of SAS (SAS Inst. Inc., Cary, NC). The model included experimental diet as a fixed variable and block, period, and pig within block as random variables. Orthogonal polynomial contrast was performed to determine linear and quadratic effects of graded concentration of casein in Exp. 1 and to determine the effects of the DDGS concentration and casein addition and their interaction in Exp. 2. Least squares means were separated by pairwise comparison with the Tukey's adjustment if interactions were observed in Exp. 2. In all statistical analyses, pig was considered as an experimental unit, and significance was declared at $P < 0.05$.

3.4 Results

All pigs were in good condition during Exp. 1 and 2 except one pig assigned to the diet containing 155.6 g/kg DDGS without casein that was removed for feed refusal during the last period of Exp. 2.

3.4.1 Chemical Analysis

The analyzed concentration of CP in DDGS and casein were 280 and 871 g/kg on as-fed basis, respectively (Table 3.2). The analyzed concentration of CP and AA in experimental diets for both Exp. 1 and 2 were similar with values calculated using the analyzed concentration of CP and AA in DDGS and casein (Table 3.3).

3.4.2 Basal Ileal Endogenous Losses of Crude Protein and Amino Acids

The BEL of CP in pigs fed the NFD was 12.9 g/kg dry matter intake (**DMI**) in Exp. 1 and 10.9 g/kg DMI in Exp. 2 (Table 3.4). In Exp. 1, the BEL of indispensable AA ranged from 86 mg/kg DMI for Met to 478 mg/kg DMI for Leu, whereas in Exp. 2, those ranged from 69 mg/kg DMI for Met to 513 mg/kg DMI for Lys. The AID of CP and AA in Exp. 1 and 2 were corrected for those BEL of CP and AA in Exp. 1 and 2.

3.4.3 Experiment 1: Amino Acid Digestibility Responses to Graded Concentration of Casein in Experimental Diets

Correction of the AID of CP and AA for BEL did not affect the observed trend in AA responses to graded concentration of casein in experimental diets, thus the AID data are not presented. The AID of CP and AA in experimental diets linearly increased ($P < 0.001$) as the concentration of casein in experimental diets increased (data not shown). Pigs fed diets containing graded concentration of casein had linear increases ($P < 0.001$) in SID of CP and AA (Table 3.5). Increasing concentration of casein in experimental diets resulted in quadratic responses ($P < 0.05$) in the AID of Lys, Ala, Cys, and Gly (data not shown). There were quadratic responses ($P < 0.05$) in the SID of Lys, Ala, Cys, and Gly in experimental diets when the concentration of casein increased. There were linear decreases ($P < 0.05$) in the SID of AA, except for Arg, Lys, Ala, Cys, and Gly, in DDGS with increasing concentration of dietary casein (Table 3.6). There was a quadratic response ($P < 0.05$) in the SID of His in DDGS as the concentration of casein in experimental diets increases. There was no effect of graded concentration of casein on SID of CP or Lys in DDGS.

3.4.4 Experiment 2: Amino Acid Digestibility Responses to Dietary Concentration of Corn Distillers' Dried Grains with Solubles with or without Casein

As in Exp. 1, correction of the AID of CP and AA for BEL did not affect the observed trend in AA responses to graded concentration of casein in experimental diets, thus the AID data are not presented. Interactions ($P < 0.01$) between the effects of DDGS concentration and casein addition were observed in the AID of CP and AA in experimental diets (data not shown). The AID of CP and most AA in the diet containing 466.8 g/kg DDGS without casein were greater ($P < 0.05$) than in the diet containing 155.6 g/kg DDGS without casein but less ($P < 0.05$) than in the diets containing DDGS and casein (data not shown). There were interactions ($P < 0.05$) between the effects of DDGS concentration and casein addition in the SID of CP and AA, except for Leu and Cys, in experimental diets (Table 3.7). Pigs fed the diet containing 466.8 g/kg DDGS without casein had greater ($P < 0.05$) SID of Arg, Ile, Lys, Met, Thr, and Val in diets than those fed the diet containing 155.6 g/kg DDGS without casein but had less ($P < 0.05$) SID values than those fed the diets containing DDGS and casein. The SID of CP in the diet containing 466.8 g/kg DDGS and 110 g/kg casein was greater ($P < 0.05$) than in the diets containing DDGS without casein but less ($P < 0.05$) than in the diet containing 155.6 g/kg DDGS and 110 g/kg casein. Pigs fed the diets containing casein had greater ($P < 0.01$) SID of CP and AA than those fed the diets without casein. The SID of AA, except for Arg and Tyr, in experimental diets was not affected by the concentration of DDGS in diets.

Interactions ($P < 0.01$) between the effects of DDGS concentration and casein addition were observed in the AID of Arg, Cys, Gly, Pro, and Tyr in DDGS (data not shown). The AID of Arg in DDGS in the diets containing 466.8 g/kg DDGS or 155.6 g/kg DDGS and 110 g/kg casein was greater ($P < 0.05$) than the value in the diet containing 155.6 g/kg DDGS without casein but less ($P < 0.05$) than the value in the diet containing 466.8 g/kg DDGS and 110 g/kg casein. The addition of casein to experimental diets increased ($P < 0.05$) the AID of CP and AA, except for Glu, in DDGS. On the other hand, pigs fed the diets containing 466.8 g/kg DDGS had greater ($P < 0.001$) AID of CP and AA than those fed the diets containing 155.6 g/kg DDGS (data not shown). There were interactions ($P < 0.05$) between the effects of DDGS concentration and casein addition in the SID of Arg, Glu, Gly, Pro, and Tyr in DDGS (Table 3.8). The SID of Arg in DDGS in the diet containing 155.6 g/kg DDGS without casein was less ($P < 0.05$) than the values in the other diets. The addition of casein in experimental diets did not affect the SID of CP and indispensable

AA, except for Arg, in DDGS. The SID of AA, except for Trp, Cys, Pro, and Ser, in DDGS in the diets containing 466.8 g/kg DDGS were less ($P < 0.05$) than the values in the diets containing 155.6 g/kg DDGS. The SID of CP and Trp in DDGS were not affected by either the concentration of DDGS or the addition of casein in experimental diets.

3.5 Discussion

The CP and indispensable AA concentrations in DDGS used in the current study are within the range of values reported in previous studies (Kim et al., 2012; NRC, 2012; Zeng et al., 2017), and those in casein are also close to the previously reported values (NRC, 2012; Brestenský et al., 2017; Park et al., 2018). The concentration of gross energy, ether extract, NDF, and ADF in DDGS are close to the values reported in previous studies (Pedersen et al., 2007; Kim et al., 2012; NRC, 2012; Zeng et al., 2017). In addition, the BEL of CP and indispensable AA in pigs estimated in Exp. 1 and 2 agree with the values in previous studies (NRC, 2012; Park et al., 2013; Adeola et al., 2016; Park et al., 2017, 2018).

The increased SID of CP and AA in diets with increasing concentration of casein are consistent with the results observed in Park et al. (2018). These observations were expected due to the greater SID of CP and AA in casein compared to DDGS. When the concentration of casein in experimental diets increased, the concentration of DDGS in diets was reduced to maintain the CP concentrations in all diets at 150 g/kg. Therefore, the concentration of digestible CP and AA in diets were increased with increasing concentration of casein in diets. The SID of CP and AA in casein are consistent with the previously reported values (Cervantes-Pahm and Stein, 2010; NRC, 2012; Park et al., 2018).

The SID of CP and AA in DDGS determined by the diet containing 466.8 g/kg DDGS without casein are within the range of the values in previous studies (Kim et al., 2012; NRC, 2012; Zeng et al., 2017). However, linearly decreasing SID of most AA in DDGS with graded concentration of casein in experimental diets were unexpected, and the reason remains unclear. The calculations for the SID of AA in DDGS from the diet containing 155.6 g/kg DDGS and 110 g/kg casein are susceptible to the errors from the low concentration of AA contributed from DDGS. Calculated indispensable AA concentrations contributed from DDGS in the diet containing 155.6 g/kg DDGS and 110 g/kg casein ranged from 0.3 g/kg for Trp to 4.8 g/kg for Leu with the mean

value of 1.8 g/kg. This reduced denominators of the equations for SID values led to the increased variations among final products of calculations.

Decreasing SID of AA in DDGS with increasing concentration of casein in experimental diets are inconsistent with the results reported in Park et al. (2018), which may be due to the differences in the treatment structure. In the experiment of Park et al. (2018), 2 isonitrogenous diets were prepared with or without the addition of 60 g/kg casein and used to compare the SID of AA in DDGS by two-sample, two-tailed *t*-test. In the current study, 3 isonitrogenous diets were prepared with graded concentration of casein, and orthogonal polynomial contrast was used to determine the effects of casein on SID of AA in DDGS. Therefore, that the positive effects of casein on SID of AA in DDGS were not detected in the current study could be due to the negative effects of dietary DDGS-associated low concentration of CP and AA.

Pigs fed the diet containing 155.6 g/kg DDGS without casein had lower SID of several indispensable AA in diets than those fed the diet containing 466.8 g/kg DDGS without casein. Liu et al. (2018) observed a linear decrease in the SID of Arg and a quadratic response in the SID of Ile with decreasing concentration of CP contributed from DDGS in experimental diets, but the SID of CP and the other AA were not affected by dietary concentration of CP. Perhaps these are due to the different lowest concentration of CP in experimental diets. Analyzed CP concentrations in diets containing the lowest concentration of DDGS were 59 and 84.7 g/kg in the current study and Liu et al. (2018), respectively. Therefore, the concentration of CP at 84.7 g/kg contributed from DDGS in experimental diets is inadequate to offset any issues with the small denominator used in calculations for the SID of AA in DDGS.

Greater SID of AA in the diet containing 155.6 g/kg DDGS and 110 g/kg casein compared with the diet containing 466.8 g/kg DDGS without casein is consistent with the results of Exp. 1. The proportion of AA contributed from casein was greater in the diet containing 155.6 g/kg DDGS than in the diet containing 466.8 g/kg DDGS. Thus, the SID of AA in the diet containing 155.6 g/kg DDGS and 110 g/kg casein were expected to be greater than the values in the diet containing 466.8 g/kg DDGS and 110 g/kg casein. Although there were no differences in the SID of most AA between the diet containing 466.8 g/kg DDGS plus 110 g/kg casein and the diet containing 155.6 g/kg DDGS plus 110 g/kg casein, the SID of AA in these 2 diets were close to the values estimated by the SID of AA in DDGS determined by the diet containing 466.8 g/kg DDGS without casein and those in casein determined in Exp. 1.

The SID of most AA in DDGS in the diets containing 155.6 g/kg DDGS were less than those in DDGS in diets containing 466.8 g/kg DDGS regardless of the addition of casein. These findings support the linear reductions in the SID of most AA in DDGS with decreasing concentration of DDGS in diets in Exp. 1. Xue et al. (2017) made observations similar to the current study that the SID of CP and AA, except for Pro, in diets containing 69 g/kg CP were less than those in diets containing 134 g/kg CP and discussed that deficiency of pigs fed diets containing low CP concentration might affect the SID of CP and AA. Therefore, it may be concluded that the low concentration of AA in experimental diets contributed from DDGS results in reduced SID of most AA in DDGS regardless of the addition of casein in diets. The addition of casein did not improve the SID of CP and most AA in DDGS, which is consistent with the results of Exp. 1. These observations may imply that the addition of casein to DDGS with low protein quality did not affect the SID of AA in DDGS. However, it is possible that the effects of casein on SID of AA in DDGS were not observed due to the reduced SID values and increased SEM in diets with low concentration of DDGS.

In conclusion, decreasing concentration of DDGS with increasing concentration of casein in experimental diets linearly decreased the SID of most AA in DDGS. In addition, low dietary concentration of CP from DDGS had reduced SID of most AA regardless of the addition of casein. Therefore, the SID of AA in DDGS may be reduced if experimental diets contain the CP concentration less than 59 g/kg. Further research is needed to verify the effects of graded concentration of casein in experimental diets on SID of CP and AA in isonitrogenous DDGS diets.

3.6 Literature Cited

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274-283. doi:10.1016/j.anifeedsci.2016.06.004

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Brestenský, M., S. Nitrayová, and P. Patráš. 2017. Ileal endogenous losses in pigs feeding a protein-free diet or diets with different contents of casein or crystalline amino acids. Arch. Anim. Nutr. 71:210-218. doi:10.1080/1745039X.2017.1312832

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674-2683. doi:10.2527/jas.2009-2677

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634. doi:10.4141/cjas79-081

Kim, B. G., D. Y. Kil, Y. Zhang, and H. H. Stein. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles fed to pigs. J. Anim. Sci. 90:3798-3808. doi:10.2527/jas2011-4692

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Liu, Z., J. Liu, L. Chen, S. Lv, L. Liu, X. Tang, and H. Zhang. 2018. Additivity of standardized ileal digestibility of amino acids in mixed diets containing multiple protein sources for growing pigs fed three crude protein levels. J. Anim. Physiol. Anim. Nutr.102:1039-1052. doi:10.1111/jpn.12912

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110-3119. doi:10.2527/jas.2017.1404

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186-192.

Park, C. S., D. Ragland, and O. Adeola. 2018. Amino acid digestibility of corn distillers' dried grains with solubles with the addition of casein in pigs. J. Anim. Sci. 96:4674-4684. doi:10.1093/jas/sky309

Pedersen, C., M. G. Boersma, and H. H. Stein. 2007. Energy and nutrient digestibility in NutriDense corn and other cereal grains fed to growing pigs. J. Anim. Sci. 85:2473-2483. doi:10.2527/jas.2006-620

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Xue, P. C., D. Ragland, and O. Adeola. 2017. Influence of dietary crude protein and phosphorus on ileal digestion of phosphorus and amino acids in growing pigs. J. Anim. Sci. 95:2071-2079. doi:10.2527/jas2016.1293

Zeng, Z. K., G. C. Shurson, and P. E. Urriola. 2017. Prediction of the concentration of standardized ileal digestible amino acids and safety margins among sources of distillers dried grains with solubles for growing pigs: A meta-analysis approach. Anim. Feed Sci. Technol. 231:150-159. doi:10.1016/j.anifeedsci.2017.07.010

Table 3.1 Ingredient composition of experimental diets containing corn distillers' dried grains with solubles (DDGS) and casein, g/kg as-fed basis

		Exp. 1[1]				Exp. 2				
	DDGS, g/kg:	466.8	311.2	155.6	0	466.8	466.8	155.6	155.6	
Item	Casein, g/kg:	0	55.0	110.0	165.0	0	110.0	0	110.0	NFD[2]
Cornstarch		354.7	439.1	523.5	607.9	354.7	247.0	630.7	523.5	768.8
DDGS		466.8	311.2	155.6	0.0	466.8	466.8	155.6	155.6	0.0
Casein		0.0	55.0	110.0	165.0	0.0	110.0	0.0	110.0	0.0
Dextrose		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil		30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Cellulose[3]		0.0	13.3	26.7	40.0	0.0	0.0	26.7	26.7	40.0
Ground limestone		13.5	12.8	12.1	11.4	13.5	14.5	11.0	12.1	10.0
Monocalcium phosphate		3.3	5.2	7.1	9.0	3.3	0.0	11.0	7.1	14.5
Salt		4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Potassium carbonate		0.0	1.3	2.7	4.0	0.0	0.0	2.7	2.7	4.0
Magnesium oxide		0.0	0.3	0.7	1.0	0.0	0.0	0.7	0.7	1.0
Vitamin premix[4]		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[5]		0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Selenium premix[6]		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide premix[7]		25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Total		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

[1]Two diets in Exp. 1 (the diet containing 466.8 g/kg DDGS without casein and the diet containing 155.6 g/kg DDGS and 110.0 g/kg casein) were also used in Exp. 2.

[2]NFD = nitrogen-free diet. Used for both Exp. 1 and 2.

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

[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; and vitamin B_{12}, 0.02 mg.

[5]Provided the following quantities per kilogram of complete diet: I, 0.258 mg; Mn, 12.0 mg; Cu, 6.33 mg; Fe, 136 mg; and Zn, 104 mg.

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

[7]Provided 5 g chromic oxide/kg of complete diet.

Table 3.2 Analyzed nutrient composition of corn distillers' dried grains with solubles (DDGS) and casein, g/kg as-fed basis

	Ingredient	
Item	DDGS	Casein
Dry matter	860	904
Gross energy, kcal/kg	4,602	5,469
Crude protein (nitrogen × 6.25)	280	871
Ether extract	106	16.3
Ash	46.9	11.9
Neutral detergent fiber	311	-
Acid detergent fiber	88.4	-
Indispensable amino acid		
Arg	12.8	27.2
His	7.4	25.0
Ile	11.5	51.3
Leu	30.9	86.1
Lys	8.8	70.6
Met	5.2	27.1
Phe	13.6	44.8
Thr	10.7	39.9
Trp	2.2	12.5
Val	14.2	61.0
Dispensable amino acid		
Ala	18.7	26.5
Asp	17.3	60.6
Cys	5.6	3.7
Glu	35.3	196.4
Gly	11.0	16.4
Pro	20.8	103.8
Ser	12.2	42.1
Tyr	10.1	45.7

Table 3.3 Analyzed concentration of dry matter, crude protein (CP), and amino acids (AA) in experimental diets containing corn distillers' dried grains with solubles (DDGS) and casein, g/kg as-fed basis

		Exp. 1[1]				Exp. 2				
	DDGS, g/kg:	466.8	311.2	155.6	0	466.8	466.8	155.6	155.6	
Item	Casein, g/kg:	0	55.0	110.0	165.0	0	110.0	0	110.0	NFD[2]
Dry matter		896	904	901	907	896	890	902	901	903
CP (nitrogen × 6.25)		148	148	152	156	148	242	59	152	20
Indispensable AA										
Arg		5.7	5.1	4.4	4.1	5.7	8.9	1.6	4.4	0.1
His		3.5	3.6	3.5	3.9	3.5	6.2	1.0	3.5	0.0
Ile		5.1	6.1	6.7	8.1	5.1	10.9	1.5	6.7	0.1
Leu		14.3	14.0	13.1	13.9	14.3	24.3	4.5	13.1	0.3
Lys		4.3	6.7	8.3	11.2	4.3	12.1	1.3	8.3	0.1
Met		2.5	3.0	3.4	3.6	2.5	5.4	0.7	3.4	0.1
Phe		6.2	6.5	6.4	7.2	6.2	11.4	1.9	6.4	0.1
Thr		4.9	5.4	5.5	6.4	4.9	9.5	1.5	5.5	0.1
Trp		1.3	1.7	1.8	2.2	1.3	2.8	0.4	1.8	0.2
Val		6.6	7.5	7.9	9.5	6.6	13.3	2.0	7.9	0.1
Dispensable AA										
Ala		9.1	7.4	5.6	4.4	9.1	12.2	2.9	5.6	0.2
Asp		8.7	9.0	8.9	9.9	8.7	15.4	2.7	8.9	0.2
Cys		2.6	2.0	1.3	0.5	2.6	3.0	0.8	1.3	0.0
Glu		19.7	23.7	25.9	31.6	19.7	40.8	6.8	25.9	0.3
Gly		5.6	4.4	3.4	2.7	5.6	7.3	1.7	3.4	0.1
Pro		10.2	12.2	13.0	16.2	10.2	21.5	3.2	13.0	0.1
Ser		5.4	5.9	6.1	7.0	5.4	10.6	1.8	6.1	0.1
Tyr		4.8	5.5	5.8	6.7	4.8	9.7	1.0	5.8	0.1

[1]Two diets in Exp. 1 (the diet containing 466.8 g/kg DDGS without casein and the diet containing 155.6 g/kg DDGS and 110.0 g/kg casein) were also used in Exp. 2.

[2]NFD = nitrogen-free diet. Used for both Exp. 1 and 2.

Table 3.4 Basal ileal endogenous losses (BEL) of crude protein (CP) and amino acids (AA) in pigs, mg/kg dry matter intake (DMI)

	Exp. 1		Exp. 2	
Item	BEL	SD	BEL	SD
CP, g/kg DMI	12.9	2.91	10.9	2.94
Indispensable AA				
Arg	432	137.3	440	166.8
His	160	25.9	143	26.6
Ile	288	39.1	269	38.7
Leu	478	67.8	414	65.5
Lys	426	72.7	513	159.3
Met	86	11.7	69	14.7
Phe	297	39.4	262	37.2
Thr	431	59.2	410	53.6
Trp	123	21.5	99	21.4
Val	439	53.0	346	49.6
Dispensable AA				
Ala	454	77.7	427	93.8
Asp	688	85.9	637	100.6
Cys	141	28.0	128	19.8
Glu	817	117.4	739	126.5
Gly	890	234.2	852	301.8
Pro	2,450	1,843.7	2,942	2,062.0
Ser	371	46.9	363	57.5
Tyr	221	26.1	204	29.8

Note: each mean represents 8 observations.

Table 3.5 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in diets containing corn distillers' dried grains with solubles (DDGS) with graded concentration of casein fed to pigs, Exp. 1

		Diet						
	DDGS, g/kg:	466.8	311.2	155.6	0		*P*-value	
Item	Casein, g/kg:	0	55.0	110.0	165.0	SEM	Linear	Quadratic
CP		77.8	85.2	91.3	98.9	1.25	< 0.001	0.948
Indispensable AA								
Arg		84.0	88.6	92.0	99.5	1.12	< 0.001	0.085
His		77.6	85.3	90.4	97.6	1.13	< 0.001	0.813
Ile		78.0	86.0	91.0	97.7	1.05	< 0.001	0.467
Leu		86.7	90.5	93.3	98.3	0.77	< 0.001	0.289
Lys		54.1	80.7	89.0	97.6	1.86	< 0.001	< 0.001
Met		85.9	91.6	95.0	99.1	0.63	< 0.001	0.167
Phe		83.3	88.9	92.4	98.1	0.87	< 0.001	0.937
Thr		71.1	80.4	86.5	95.0	1.28	< 0.001	0.722
Trp		81.7	88.7	92.3	97.5	1.39	< 0.001	0.335
Val		77.3	85.2	90.4	97.3	1.09	< 0.001	0.619
Dispensable AA								
Ala		82.5	85.8	87.8	96.9	1.21	< 0.001	0.004
Asp		70.1	80.0	86.8	96.9	1.32	< 0.001	0.945
Cys		72.3	75.1	76.3	85.1	1.74	< 0.001	0.026
Glu		84.7	90.4	93.7	98.1	0.78	< 0.001	0.356
Gly		71.7	79.1	86.0	105.3	2.56	< 0.001	0.003
Pro		95.4	102.3	106.6	106.7	2.60	< 0.001	0.099
Ser		77.8	83.6	89.1	96.8	0.99	< 0.001	0.282
Tyr		86.2	91.5	95.1	98.9	0.61	< 0.001	0.171

Note: each least squares mean represents 8 observations.

Table 3.6 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in corn distillers' dried grains with solubles (DDGS) with graded concentration of casein fed to pigs, Exp. 1

		Diet[1]					
	DDGS, g/kg:	466.8	311.2	155.6		*P*-value	
Item	Casein, g/kg:	0	55.0	110.0	SEM	Linear	Quadratic
CP		77.6	77.8	74.4	2.57	0.188	0.374
Indispensable AA							
Arg		83.8	84.8	80.7	2.09	0.112	0.133
His		77.1	77.9	71.3	2.34	0.014	0.048
Ile		77.6	76.7	68.3	2.72	0.004	0.119
Leu		86.4	86.7	82.6	1.70	0.022	0.102
Lys		52.5	57.1	38.9	5.46	0.056	0.061
Met		85.6	84.7	78.8	1.84	0.003	0.150
Phe		82.9	83.6	77.8	1.99	0.014	0.056
Thr		70.6	70.9	62.2	3.44	0.033	0.168
Trp		81.6	79.6	66.0	4.14	0.002	0.140
Val		76.9	76.0	67.4	2.90	0.005	0.145
Dispensable AA							
Ala		82.3	83.1	78.2	2.14	0.062	0.120
Asp		69.7	69.7	60.4	3.32	0.018	0.137
Cys		72.2	74.0	71.5	2.39	0.732	0.219
Glu		84.6	82.9	76.0	1.97	< 0.001	0.143
Gly		71.6	72.2	65.7	3.98	0.226	0.393
Pro		96.1	97.8	104.8	3.37	0.022	0.371
Ser		77.8	75.6	69.8	2.44	0.012	0.475
Tyr		85.8	85.6	81.9	1.27	0.010	0.118

Note: each least squares mean represents 8 observations.

[1]Standardized ileal digestibility of CP and AA in DDGS determined by the diets containing casein were calculated by the difference method with the standardized ileal digestibility of CP and AA in casein.

Table 3.7 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in diets containing corn distillers' dried grains with solubles (DDGS) with or without the addition of casein fed to pigs, Exp. 2

		Diet							
	DDGS, g/kg:	466.8	466.8	155.6	155.6		*P*-value[1]		
Item	Casein, g/kg:	0	110.0	0	110.0	SEM	DDGS	Casein	D × C
CP		73.6^{c}	84.7^{b}	73.7^{c}	90.5^{a}	1.43	0.011	< 0.001	0.015
Indispensable AA									
Arg		79.7^{b}	88.9^{a}	69.0^{c}	91.6^{a}	2.15	0.011	< 0.001	< 0.001
His		71.7^{b}	85.3^{a}	66.9^{b}	89.1^{a}	1.64	0.721	< 0.001	0.002
Ile		71.3^{b}	85.6^{a}	63.8^{c}	89.4^{a}	1.57	0.188	< 0.001	< 0.001
Leu		82.9	90.7	80.6	92.3	1.05	0.714	< 0.001	0.050
Lys		41.4^{b}	80.0^{a}	24.7^{c}	87.8^{a}	2.99	0.106	< 0.001	< 0.001
Met		79.0^{b}	90.6^{a}	72.4^{c}	93.7^{a}	1.23	0.154	< 0.001	< 0.001
Phe		78.3^{b}	89.3^{a}	74.5^{b}	91.3^{a}	1.22	0.456	< 0.001	0.015
Thr		64.0^{b}	81.2^{a}	57.5^{c}	85.6^{a}	1.73	0.504	< 0.001	0.001
Trp		77.9^{b}	88.4^{a}	74.7^{b}	92.9^{a}	1.74	0.713	< 0.001	0.038
Val		72.8^{b}	86.3^{a}	66.9^{c}	89.7^{a}	1.46	0.329	< 0.001	0.001
Dispensable AA									
Ala		77.7^{b}	85.2^{a}	74.3^{b}	86.3^{a}	1.38	0.302	< 0.001	0.042
Asp		62.6^{b}	80.3^{a}	58.5^{b}	85.5^{a}	1.84	0.726	< 0.001	0.004
Cys		67.8	72.0	65.7	74.5	1.53	0.896	< 0.001	0.099
Glu		80.4^{b}	89.6^{a}	79.7^{b}	92.8^{a}	0.99	0.173	< 0.001	0.042
Gly		65.5^{b}	77.7^{a}	48.7^{c}	85.4^{a}	3.92	0.151	< 0.001	< 0.001
Pro		90.6^{ab}	100.8^{a}	52.7^{b}	108.0^{a}	12.82	0.148	0.004	0.039
Ser		72.2^{b}	83.5^{a}	70.1^{b}	87.7^{a}	1.53	0.437	< 0.001	0.023
Tyr		81.9^{b}	92.0^{a}	69.3^{c}	94.1^{a}	1.08	< 0.001	< 0.001	< 0.001

Note: each least squares mean represents 8 observations except for the diet containing 155.6 g/kg DDGS without casein (7 observations).

$^{a\text{-}c}$Means within a row with different superscripts differ ($P < 0.05$).

[1]D × C = interaction between the effects of dietary DDGS concentration and casein addition.

Table 3.8 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in corn distillers' dried grains with solubles (DDGS) with or without the addition of casein fed to pigs, Exp. 2

		Diet[1]							
	DDGS, g/kg:	466.8	466.8	155.6	155.6		*P*-value[2]		
Item	Casein, g/kg:	0	110.0	0	110.0	SEM	DDGS	Casein	D × C
CP		73.6	74.1	73.5	71.8	2.23	0.421	0.690	0.441
Indispensable AA									
Arg		79.7[a]	83.6[a]	68.7[b]	79.6[a]	2.90	< 0.001	< 0.001	0.044
His		71.7	75.1	66.7	67.5	2.70	0.002	0.247	0.495
Ile		71.3	72.5	63.5	61.8	2.71	< 0.001	0.899	0.505
Leu		82.9	85.5	80.5	79.7	1.43	0.001	0.441	0.134
Lys		41.4	48.0	24.3	29.7	4.29	< 0.001	0.115	0.869
Met		79.0	80.2	72.2	72.6	1.63	< 0.001	0.586	0.807
Phe		78.3	82.1	74.4	74.5	1.71	< 0.001	0.159	0.189
Thr		64.0	68.5	57.2	59.3	3.07	0.002	0.167	0.596
Trp		77.8	76.6	73.8	71.7	3.44	0.123	0.568	0.879
Val		72.8	74.6	66.6	65.1	2.24	< 0.001	0.933	0.329
Dispensable AA									
Ala		77.7	81.1	74.2	75.3	1.82	0.002	0.105	0.411
Asp		62.6	66.2	58.1	56.0	3.17	0.003	0.737	0.210
Cys		67.8	69.9	65.6	69.3	1.72	0.357	0.057	0.595
Glu		80.4[a]	78.3[a]	79.5[a]	71.3[b]	1.89	0.013	0.002	0.049
Gly		65.5[a]	68.0[a]	48.5[b]	64.4[a]	4.27	0.004	0.010	0.049
Pro		91.3[ab]	93.3[ab]	52.4[b]	109.3[a]	13.03	0.278	0.011	0.016
Ser		72.2	72.5	69.8	65.3	3.08	0.072	0.427	0.370
Tyr		81.9[ab]	84.3[a]	69.2[c]	77.9[b]	1.63	< 0.001	< 0.001	0.025

Note: each least squares mean represents 8 observations except for the diet containing 155.6 g/kg DDGS without casein (7 observations).

[a-c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Standardized ileal digestibility of CP and AA in DDGS determined by the diets containing casein were calculated by the difference method with the standardized ileal digestibility of CP and AA in casein.

[2]D × C = interaction between the effects of dietary DDGS concentration and casein addition.

CHAPTER 4. COMPARISON OF AMINO ACID DIGESTIBILITY IN FULL-FAT SOYBEAN, TWO SOYBEAN MEALS, AND PEANUT FLOUR BETWEEN BROILER CHICKENS AND GROWING PIGS

A version of this chapter has been published in Journal of Animal Science.

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110–3119. doi:10.2527/jas.2017.1404

4.1 Abstract

The aim of this study was to determine the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in full-fat soybean (FFSB), solvent-extracted soybean meal containing 430 g/kg CP (SBM-43), solvent-extracted soybean meal containing 470 g/kg CP (SBM-47), and peanut flour (PNF) fed to broiler chickens and pigs, and to compare the digestibility of CP and AA between broiler chickens and pigs. Four diets were prepared to contain FFSB, SBM-43, SBM-47, and PNF, respectively, as the sole source of nitrogen. A nitrogen-free diet was formulated to estimate the basal ileal endogenous losses of CP and AA for broiler chickens and pigs. In Exp. 1, a total of 416 twenty-one-d-old male broiler chickens [initial body weight (BW) = 922.1 ± 79.9 g] were assigned to 5 experimental diets in a randomized complete block design with BW as a blocking factor. After 5 d of adaptation, ileal digesta samples were collected after birds were euthanized by CO_2 asphyxiation. In Exp. 2, twenty barrows (initial BW = 62.0 ± 6.9 kg) surgically fitted with T-cannulas at the distal ileum were allotted to 5 experimental diets with 2 consecutive 7-d experimental periods. After 5 d of adaptation, ileal digesta samples were collected at d 6 and 7. For statistical analysis, treatments were considered a 2 × 4 factorial arrangement with effects of species and experimental diets (Exp. 1 vs. 2). There were no interactions between species and diets for the digestibility of CP and AA except for Cys ($P < 0.01$). The AID of CP and indispensable AA in pigs were greater ($P < 0.01$) than in broiler chickens. In both broiler chickens and pigs, the AID of CP and indispensable AA in SBM-47 were greater ($P < 0.05$) than in FFSB, and the AID of Lys in PNF was the least ($P < 0.05$) among ingredients. The SID of CP and indispensable AA in pigs were greater ($P < 0.05$) than in broiler chickens except for Trp. In both broiler chickens and pigs, the SID of Lys in PNF

was the least ($P < 0.05$) among ingredients. In broiler chickens, the SID of CP, Ile, Leu, Thr, Trp, and Val in FFSB were less ($P < 0.05$) than those in SBM-43, SBM-47, and PNF. In pigs, the SID of Arg, Ile, Leu, Met, Phe, and Val in FFSB were less ($P < 0.05$) than in SBM-43, SBM-47, and PNF. In conclusion, the digestibility of CP and most AA were less in broiler chickens than in pigs, but the pattern of differences in the AA digestibility among ingredients was similar between broiler chickens and pigs.

Key words: amino acid digestibility, broiler chickens, full-fat soybean, peanut flour, pigs, soybean meal

4.2 Introduction

Chickens and pigs are the dominant nonruminant animals in the livestock industry. Due to the similarity of their digestive systems, most of the terminologies and experimental procedures to determine the nutrient availability are similar between chickens and pigs. Standardized ileal digestibility (**SID**) has been widely used for both chickens and pigs to evaluate the amino acid (**AA**) availability of feedstuffs (Kong and Adeola, 2014). However, although their digestive systems are similar, the SID of AA in chickens and pigs fed the same feedstuffs may be different from each other due to the differences in digestive organs such as difference between gizzard of chickens and stomach of pigs (Pond et al., 2005).

Full-fat soybean (**FFSB**) is a valuable feed ingredient in both poultry and swine diets due to the high concentration of crude protein (**CP**) and energy (Ravindran et al., 2014a; Woyengo et al., 2014); however, because raw FFSB contains considerable concentration of trypsin inhibitors, FFSB is typically used in animal diets after heat processing (Waldroup, 1982). During the production of soybean meal (**SBM**) after oil extraction from FFSB, heat is also applied to reduce trypsin inhibitors. However, the effects of heat processing on AA digestibility in SBM may be different from FFSB, and AA digestibility of SBM may also be affected by the inclusion rate of soybean hulls in SBM. Peanut flour (**PNF**) is produced by dehulling and grinding of peanuts. Because PNF is high in CP (Iyayi and Adeola, 2014), it may be a valuable protein source for both chickens and pigs; however, information for AA digestibility is scarce. Therefore, the objectives of this experiment were to determine the apparent ileal digestibility (**AID**) and SID of CP and AA in FFSB, hulled SBM containing 430 g/kg CP (**SBM-43**), dehulled SBM containing 470 g/kg CP

(**SBM-47**), and PNF fed to broiler chickens and pigs and to compare the digestibility of CP and AA between broiler chickens and pigs.

4.3 Materials and Methods

Protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

4.3.1 Ingredients and Experimental Diets

The FFSB originated from soybeans that were dehulled and extruded (Table 4.1). The SBM-43 and SBM-47 originated from solvent extraction processes of soybeans with and without hulls, respectively. Different soybeans were used for FFSB, SBM-43, and SBM-47. The PNF was obtained by dehulling and grinding of peanuts. Four diets were formulated to contain FFSB, SBM-43, SBM-47, and PNF as the sole source of nitrogen (**N**), all of which were prepared to contain the identical concentration of CP at 150 g/kg as-fed basis (Table 4.2). A N-free diet (**NFD**) was also formulated to estimate the basal ileal endogenous losses (**BEL**) of CP and AA for broiler chickens and pigs. All diets were formulated to meet or exceed the vitamin and mineral requirement estimates for both broiler chickens [National Research Council (**NRC**), 1994] and pigs (NRC, 2012). Chromic oxide was added at 5 g/kg of diet as an indigestible index.

4.3.2 Experiment 1: Digestibility of Amino Acids for Broiler Chickens

A total of 416 male broiler chicks (Ross 708, Aviagen, Huntsville, AL) were obtained from a commercial hatchery. The mean body weight (**BW**) at d 0 after hatching was 40.6 g. Birds were weighed and tagged at d 0, and raised in an electrically heated battery brooder (model SB 4 T, Alternative Design Manufacturing & Supply, 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 25, respectively. Birds were fed a corn-SBM-based starter diet containing 210 g/kg CP from d 0 to 20. On d 21, broiler chickens were individually weighed and assigned to 5 experimental diets in a randomized complete block design with BW as a blocking factor using a spreadsheet program (Kim and Lindemann, 2007). The mean initial BW was 922.1 g (SD = 79.9). Each treatment contained 8 replicate cages and each cage contained 10 birds except for the NFD treatment which contained 12 birds per cage.

Birds were fed the assigned experimental diets from d 21 to 25. Birds had free access to feed and water during the overall experimental period. On d 26, birds were euthanized by CO_2 asphyxiation, weighed individually, and dissected for ileal digesta collection. Ileal digesta in the distal ileum, from Meckel's diverticulum to approximately 20 mm proximal to the ileocecal junction, was flushed from the intestine by distilled water and pooled within cages as previously described (Kong and Adeola, 2013a). Collected ileal digesta samples were stored at –20°C before further analyses.

4.3.3 Experiment 2: Digestibility of Amino Acids for Pigs

Twenty barrows (initial BW 62.0 ± 6.9 kg) surgically fitted with T-cannulas at the distal ileum as described by Dilger et al. (2004) were used. Pigs were allotted to 4 blocks based on BW and assigned to a quadruplicate 5 × 2 incomplete Latin Square design with 5 dietary treatments and 2 periods using a spreadsheet program (Kim and Kim, 2010). Pigs were housed in metabolism crates equipped with a feeder and a nipple drinker. Water was available at all times.

During the experimental periods, pigs received their assigned daily feed allowance, which was calculated as 4% of BW of the lightest pig in each block, divided into 2 equal meals, and fed at 0700 and 1700 h. There were 5 d of adaptation and 2 d of ileal digesta collection in each experimental period. On d 6 and 7 of the experimental period, plastic sample bags (Whirl-Pak bag, NASCO, Fort Atkinson, WI) containing 10 mL of 10% formic acid were attached to T-cannulas from 0730 to 1700 h. Attached bags were inspected every 30 min and changed whenever they were filled. Collected ileal digesta samples were immediately stored at –20°C. After each experimental period, ileal digesta samples were slightly thawed and pooled within pigs and diets.

4.3.4 Chemical Analysis

Ileal digesta samples from birds and pigs were lyophilized before the chemical analyses. Ingredients, experimental diets, and ileal digesta samples were finely ground using a coffee grinder. The concentration of dry matter (**DM**) in ingredients, diets, and ileal digesta samples were measured by drying at 105°C for 24 h in a forced-air oven [Precision Scientific Co., Chicago, IL; method 934.01; Association of Official Analytical Chemists (**AOAC**), 2006]. Ingredients were analyzed for gross energy (**GE**) by an isoperibol bomb calorimeter (Model 6200, Parr Instrument Co., Moline, IL), acid hydrolyzed ether extract (**AEE**; method 954.02; AOAC, 2000), and ash

(method 942.05; AOAC, 2006). Crude fiber (method 978.10; AOAC, 2006), neutral detergent fiber (Van Soest et al., 1991), and acid detergent fiber [method 973.18 (AD); AOAC, 2006] were analyzed by a fiber analyzer (ANKOM 200 Fiber Analyzer, ANKOM Technology, Macedon, NY). Trypsin inhibitor activity (**TIA**) in feed ingredients was analyzed [method Ba 12-75; American Oil Chemists' Society (**AOCS**), 2011] as trypsin inhibitor units (**TIU**) per mg of sample. Urease activity (method Ba 9-58; AOCS, 2011) and protein solubility (Araba and Dale, 1990) for ingredients were also analyzed. The concentration of N in ingredients, diets, and ileal digesta samples were analyzed by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the concentration of CP was calculated by multiplying 6.25 by the concentration of N. For analysis of AA concentrations in ingredients, diets, and ileal digesta samples, samples were hydrolyzed in 6 M HCl (or BaOH for the analysis of Trp) at 110°C for 24 h under N atmosphere. Performic acid oxidation was conducted before acid hydrolysis to analyze the concentration of Met and Cys. High-performance liquid chromatography after post-column derivatization was used to determine the AA concentration in hydrolyzed samples [method 982.30 E (a, b, c); AOAC, 2006], which was conducted by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Chromium concentration in diets and ileal digesta samples were measured by a spectrophotometer at 450 nm of absorption (Spectronic 21D; Milton Roy Co., Rochester, NY) after wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

4.3.5 Calculations and Statistical Analysis

The AID (%), BEL (g/kg DM intake), and SID (%) of CP and AA were calculated based on the equations suggested in Kong and Adeola (2014):

$$\text{AID (\%)} = [1 - (Cr_i/Cr_o) \times (AA_o/AA_i)] \times 100,$$

$$\text{BEL (g/kg DM intake)} = AA_o \times (Cr_i/Cr_o),$$

$$\text{SID (\%)} = \text{AID} + (\text{BEL}/AA_i) \times 100,$$

in which Cr_i and Cr_o represent the concentration of Cr (g/kg DM) in diets and ileal digesta, respectively; AA_i and AA_o represent the concentration of CP or AA (g/kg DM) in diets and ileal digesta, respectively.

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the species, experimental diets, diets × species interactions, and block

within species as independent variables. To coordinate the effect of block in the model, the effect of period in Exp. 2 was separated into blocks. Therefore, the same block in the different period was regarded as a different block (i.e., total 8 blocks), and the effect of animal in Exp. 2 was excluded. The PDIFF option with the Tukey's adjustment was used within species to separate the least squares means for dietary treatments. Simple *t* tests were conducted by TTEST procedure of SAS to compare the BEL of CP and AA between broiler chickens and pigs. The experimental unit was the cage for Exp. 1 and the pig for Exp. 2, and statistical significance was declared at $P < 0.05$.

4.4 Results

During the experimental periods, all animals were in a good condition except for 2 pigs in Exp. 2, which lost their T-cannulas during the second period. Those pigs were assigned to be fed the NFD and the diet containing PNF, respectively. Therefore, there were 7 observations for the BEL of CP and AA and the digestibility of CP and AA in PNF.

4.4.1 Chemical Analysis

The concentration of CP in FFSB, SBM-43, SBM-47, and PNF were 358, 433, 467, and 466 g/kg as-fed basis, respectively (Table 4.1). The concentration of AEE ranged from 29.3 g/kg in SBM-47 to 259.4 g/kg in PNF. The analyzed TIA were 5.40, 2.40, 3.40, and 1.35 TIU/mg in FFSB, SBM-43, SBM-47, and PNF, respectively. Values for the urease activity in FFSB, SBM-47, and PNF were 0.02 or less than 0.02, which was the value for the limit of quantification. The concentration of Lys in test ingredients ranged from 11.7 g/kg for PNF to 30.7 g/kg for SBM-47. The concentrations of CP in experimental diets were close to the targeted value of 150 g/kg as-fed basis (Table 4.3). The analyzed AA concentrations in the experimental diets were comparable with the calculated values based on the concentrations of AA in the test ingredients.

4.4.2 Apparent Ileal Digestibility of Crude Protein and Amino Acids

There were no interactions for AID of CP and AA between species and experimental diets except for Cys ($P = 0.002$; Table 4.4). In broiler chickens, the AID of Cys in PNF was greater ($P < 0.05$) than that in FFSB and SBM-47, and was not different from SBM-43; however, in pigs, the AID of Cys in PNF was not different from the other ingredients, and the AID of Cys in SBM-43

and SBM-47 were greater ($P < 0.05$) than in FFSB. Except for Ala, Gly, and Pro, the AID of CP and AA in pigs were greater ($P < 0.05$) than in broiler chickens. In broiler chickens, the AID of CP, Ile, Leu, and Val in FFSB were less ($P < 0.05$) than in SBM-43, SBM-47, and PNF. In broiler chickens, the AID of Lys in PNF was less ($P < 0.05$) than in the other ingredients, and the SID of Lys in SBM-47 was greater ($P < 0.05$) than in FFSB but was not different from SBM-43. The AID of His, Met, Thr, and Trp in birds fed FFSB were less ($P < 0.05$) than in those fed SBM-43 and SBM-47 but were not different from birds fed PNF. In pigs, the AID of Arg, Ile, Leu, Met, Phe, and Val in FFSB were less ($P < 0.05$) than in SBM-43, SBM-47, and PNF. The AID of CP and Thr in pigs fed FFSB was less ($P < 0.05$) than pigs fed SBM-43 and SBM-47 but were not different from values determined in pigs fed PNF. Similar with the AID of Lys in broiler chickens, the AID of Lys in pigs fed PNF was less ($P < 0.05$) than in pigs fed the other ingredients, and the SID of Lys in SBM-47 was greater ($P < 0.05$) than in FFSB but was not different from SBM-43.

4.4.3 Standardized Ileal Digestibility of Crude Protein and Amino Acids

The BEL of CP and AA for broiler chickens were not different from those for pigs except for Cys, Gly, and Pro (Table 4.5). The BEL of Cys for broiler chickens was greater ($P = 0.003$) than for pigs, but the BEL of Gly and Pro for broiler chickens were less ($P < 0.01$) than pigs.

Similar to the AID values, an interaction ($P = 0.001$) between species and diets was observed for the SID of Cys; however, interactions were not observed among the SID of CP and the other AA (Table 4.6). In broiler chickens, the SID of Cys in PNF was greater ($P < 0.05$) than that in FFSB and SBM-47 but was not different from that in SBM-43. Pigs fed FFSB had reduced ($P < 0.05$) SID of Cys compared with pigs fed SBM-43, SBM-47, or PNF. The SID of CP and AA, except for Trp, Ala, and Glu, in pigs were greater ($P < 0.05$) than those in broiler chickens. In broiler chickens, the SID of CP, Ile, Leu, Thr, Trp, and Val in FFSB were less ($P < 0.05$) than in the other ingredients. Broiler chickens fed PNF had reduced ($P < 0.05$) SID of Lys compared with those fed the other ingredients, and the SID of Lys in broiler chickens fed SBM-47 was greater ($P < 0.05$) than in FFSB, but not different from SBM-43. The SID of His and Met in birds fed FFSB was less ($P < 0.05$) than SBM-43 and SBM-47 but was not different from PNF. The SID of Phe in PNF was greater ($P < 0.05$) than that in FFSB and SBM-43 but was not different from that in SBM-47. The SID of Arg in birds fed FFSB was less ($P < 0.05$) than in those fed SBM-47 or PNF but was not different from birds fed SBM-43. In pigs, the SID of Arg, Ile, Leu, Met, Phe, and Val

in FFSB was less ($P < 0.05$) than in SBM-43, SBM-47, and PNF. The SID of CP and Thr in pigs fed FFSB was less ($P < 0.05$) than pigs fed SBM-43 and SBM-47 but was not different from PNF. Pigs fed SBM-47 had greater ($P < 0.05$) SID of His and Lys compared with pigs fed FFSB and PNF, but those values were not different from pigs fed SBM-43. The SID of Trp in pigs fed SBM-43 and SBM-47 was greater ($P < 0.05$) than in pigs fed FFSB or PNF. In pigs, the SID of Phe in SBM-43 and SBM-47 was greater ($P < 0.05$) than in FFSB, but less ($P < 0.05$) than in PNF.

4.5 Discussion

The concentration of CP and AA in FFSB were within the range of previously reported values (NRC, 2012; Iyayi and Adeola, 2014; Ravindran et al., 2014a). As expected, the concentration of CP and AA in SBM-43 were slightly less than those in SBM-47 because the CP and AA contents in SBM were diluted by the inclusion of soybean hulls. Analyzed concentration of CP and AA were close to previously reported values for SBM-43 (Sauvant et al., 2004; NRC, 2012; Kong and Adeola, 2013a) and for SBM-47 (NRC, 2012; Ravindran et al., 2014b; Liu et al., 2016). Information for PNF was scarce but the analyzed CP and AA concentration agreed with a previous study (Iyayi and Adeola, 2014). The concentration of CP in PNF was comparable with SBM-47. However, PNF had greater concentrations of GE and AEE compared with SBM-47 due to the greater oil content in peanuts (Sheppard and Rudolf, 1991). The TIA in FFSB was within the range of values suggested in previous studies which ranged from 2.74 to 5.70 TIU/mg (Baker et al., 2010; Goebel and Stein, 2011; Ravindran et al., 2014a; Woyengo et al., 2014). Values for the TIA and protein solubility in SBM-43 and SBM-47 were less than for FFSB. The TIA in SBM-47 was above the range in 16 samples of SBM that originated from the United States (2.02 to 2.96 TIU/mg; Ravindran et al., 2014b). The reason for these results may be due to the differences in temperature during heat production. Heger et al. (2016) reported that increased temperature and time during conditioning or expanding of FFSB decreased the TIA, urease activity, and protein solubility. Urease activity and protein solubility have been used to evaluate the adequacy of processing (Araba and Dale, 1990). However, protein solubility of FFSB used in the current experiment was comparable with that of uncooked FFSB used in Herkelman et al. (1991) and was greater than in FFSB used in Valencia et al. (2009) and Ravindran et al. (2014a). Therefore, this observation may indicate that the heat processing for FFSB may not be enough to maximize its AA availability.

Except for Cys, there were no interactions on the AID and SID of CP and AA between species and ingredients; however, the AID and SID of CP and most AA in pigs were greater than in broiler chickens. This observation may indicate that the pattern of differences in AA digestibility among ingredients was similar between broiler chickens and pigs, although the digestive capacity of pigs is greater than in broiler chickens. A similar observation was reported by Skrede et al. (1998) that the AID of AA in bacterial protein meal for pigs was correlated with the apparent total tract digestibility of AA in that for Leghorn roosters. The reason for greater AA digestibility for pigs compared with broiler chickens may be related on the passage rate of digesta. Kim et al. (2007) reported that the passage rate of digesta ranged from 12 to 80 h in growing pigs, and the digestibility of DM was correlated with the passage rate of digesta in pigs. However, Rochell et al. (2012) reported that mean retention time of digesta in broiler chickens was 5.62 h when fed corn-SBM-based diet containing 50 g/kg meat and bone meal. Although there is a large variation in passage rate of digesta within animals in both pigs (Kim et al., 2007) and broiler chickens (Rochell et al., 2012), it is evident that pigs have slower passage rate than broiler chickens due to the anatomical differences in the gastrointestinal tract. In addition to the passage rate, the length of the small intestine may also affect the digestibility of AA. Due to the longer small intestine of pigs compared with broiler chickens, pigs may have a greater capacity to digest and absorb AA because AA digestion and absorption mostly occur in the small intestine. However, in terms of the relative length to the BW, pigs have a shorter small intestine compared with broiler chickens. Adeola and King (2006) reported that the relative length of the small intestine of pigs with the BW of 23.32 kg was 58.23 cm/kg BW. However, according to Tahmasebi and Toghyani (2016), the relative length of the small intestine of 11 d old broiler chickens with the BW of 212.8 g was 454.9 cm/kg BW. Therefore, in consideration of other factors affecting the digestive capacity such as viscosity or digestive enzyme activity, it may be concluded that pigs had the greater digestibility of AA in test ingredients used in this experiment compared with broiler chickens. Further research is needed to determine the factors affecting the digestibility of AA for pigs and broiler chickens, and to clarify whether pigs have greater digestibility of AA in most of feed ingredients than broiler chickens.

There were no differences in the BEL of CP and indispensable AA between broiler chickens and pigs, although pigs showed the greater AID and SID of CP and most AA compared with broiler chickens. The BEL of CP and indispensable AA for broiler chickens observed in this

experiment were within the range of previous reports (Kong and Adeola, 2013a; Adedokun et al., 2014; Ravindran et al., 2014a). However, the BEL of CP and indispensable AA for pigs were relatively less than previous reports (NRC, 2012; Park et al., 2013; Xue et al., 2014; Liu et al., 2016) especially for Trp, which was less than the minimum value from the summary of 30 results reported by Park et al. (2013). The reason for this observation remains unclear; however, it may be speculated that the BEL of CP and AA observed in this experiment were affected by the ingredient composition of NFD, which was different from previous studies. In the experiment of Xue et al. (2014) and Liu et al. (2016), NFD was formulated based on cornstarch and 100 g dextrose/kg and 200 g sucrose/kg, respectively. However, in this experiment, NFD was prepared mainly based on dextrose. Due to this potential effect, previous reviews suggested the standard formulation for NFD (Stein et al., 2007; Kong and Adeola, 2014). However, Kong et al. (2014) reported that there were no differences in the BEL of CP and most AA for pigs fed 5 NFD with different ratios of cornstarch to dextrose.

Values for the SID of CP and AA in FFSB concurred with the values in previous studies for broiler chickens (Iyayi and Adeola, 2014; Ravindran et al., 2014a) and pigs (NRC, 2012). In both broiler chickens and pigs, the SID of CP and most AA in FFSB was less than those in SBM-43, SBM-47, and PNF. This may be due to the greater value of the TIA in FFSB compared with the other ingredients. Palliyeguru et al. (2011) reported that the AID of CP in broiler chickens fed corn-SBM-based diets containing 300 g/kg FFSB decreased linearly as increasing value of TIA in diets from 3.61 to 16.1 TIU/mg. Valencia et al. (2009) also reported that the SID of CP, Leu, Met, Val, and Ala in broiler chickens fed FFSB were less than broiler chickens fed SBM (452 g/kg CP); however, there were no differences in the SID of the remaining AA between FFSB and SBM, and the SID of CP and most AA in FFSB were greater than those in FFSB determined in this experiment. In previous research with pigs, Goebel and Stein (2011) reported that there were no differences in the SID of CP and AA among pigs fed either heat-treated FFSB or SBM containing 474.7 g/kg CP despite of the greater value for TIA in FFSB. The SID of CP and most AA in heat-treated FFSB reported in Goebel and Stein (2011) were greater than the results of this experiment. Moreover, Baker et al. (2010) reported that pigs fed a conventional variety of FFSB showed greater SID of CP, Leu, Lys, Met, and Phe compared with pigs fed SBM (487 g/kg CP), but the TIA in the conventional variety of FFSB was greater than in SBM. Woyengo et al. (2014) reported that the SID of the indispensable AA except for Trp in pigs fed micronized FFSB were less than those

in pigs fed SBM (471 g/kg CP) but the TIA value in FFSB was less than in SBM. These discrepancies among studies may be due to differences in processing or variety of FFSB or both. One of the most important factors in processing FFSB is heating time during steaming or extruding. Herkelman et al. (1991) reported that weight gain and feed efficiency of broiler chickens fed diets containing 371.8 g/kg FFSB quadratically increased as increasing time of autoclaving FFSB from 0 to 90 min at 105°C but birds fed the FFSB that had been autoclaved for more than 40 min had reduced weight gain and feed efficiency. In addition to heating time, variety may be responsible for the differences in results among studies. Qin et al. (1998) observed that FFSB that originated from China had greater TIA and residual TIA after heating at various temperatures and times compared with FFSB that originated from Argentina, which may lead to differences in AA digestibility.

It is well known that increased concentration of fiber in diets has negative effects on digestibility of nutrients and growth performance of both broiler chickens and pigs. Walugembe et al. (2014) reported that broiler chickens fed corn-SBM-based diets containing 60 to 80 g/kg distillers dried grains with solubles and 60 to 80 g/kg wheat bran showed decreased average daily gain (**ADG**) compared with broiler chickens fed corn-SBM-based diets without distillers dried grains with solubles or wheat bran in a 21-d growth performance trial, but there was no difference in N-corrected apparent metabolizable energy among dietary treatments. Dilger et al. (2004) reported that the SID of Arg, His, Lys, and Phe in pigs fed semi-purified diets containing SBM and soy hulls linearly decreased as increasing concentration of soy hulls from 0 to 90 g/kg. However, in this experiment, the SID of CP and AA in SBM-43 were not different from those in SBM-47 when fed to broiler chickens and pigs. Differences in results from Dilger et al. (2004) may be due to the differences in treatment structure and consequent statistical analysis. When comparing values between the 2 studies, the SID of CP and AA in SBM-43 and SBM-47 observed in this experiment agreed with the SID values of dehulled SBM with 60 g/kg soy hulls and dehulled SBM without soy hulls, respectively (Dilger et al., 2004). Therefore, it is concluded that inclusion of soy hulls to SBM up to the level to decrease the CP concentration as 430 g/kg did not affect the SID of CP and AA in dehulled SBM. Park et al. (2014) also reported that the SID of indispensable AA in dehulled SBM that originated from Korea were not different from those in hulled SBM that originated from Korea and India. In both SBM-43 and SBM-47, the SID of Cys for broiler chickens was less than the values reported in previous studies (Adedokun et al., 2009; Kong and Adeola,

2013a, b; Ravindran et al., 2014b). The reason for this observation remains unclear. There might be an unidentified analytical error when analyzing Cys in ileal digesta samples for broiler chickens. However, except for Cys, the SID of CP and remaining AA for broiler chickens agreed with previously reported values for SBM-43 (Kong and Adeola, 2013a) and for SBM-47 (Adedokun et al., 2009; Kong and Adeola, 2013b; Ravindran et al., 2014b). The SID of CP and AA for pigs also agreed with previously reported values for SBM-43 (Sauvant et al., 2004; NRC, 2012) and for SBM-47 (NRC, 2012; Xue et al., 2014; Liu et al., 2016).

Peanuts have been mostly used in poultry and swine diet as peanut meal (Batal et al., 2005; Li et al., 2014), which is the byproduct of peanuts after oil extraction. However, due to the high concentration of oil and CP in peanuts (Sheppard and Rudolf, 1991), whole peanuts can also be used as feed ingredient as both energy and AA sources. In this experiment, whole peanuts were dehulled and ground to be used as a feed ingredient. In both broiler chickens and pigs, the SID of CP and most AA in PNF were not different from those in SBM-43 and SBM-47 but the SID of Lys in PNF was less than in the other ingredients. The reason for the lower SID of Lys in PNF compared with the other ingredients remains unclear; however, it may be speculated that there was an antagonistic effect of Arg on digestibility of Lys. Pond et al. (2005) reported that Arg and Lys have the same transport system in the small intestine, and Arg inhibits the transport of Lys. However, D'Mello and Lewis (1970) reported that the excessive intake of Lys decreased ADG and gain-to-feed ratio of broiler chickens fed diets containing adequate amount of Arg, which was opposite to the current experiment because the concentration of Arg in PNF was approximately 5 times greater than that of Lys. In pigs, the excessive intake of Arg decreased the ADG, average daily feed intake, and plasma AA concentrations but did not affect gain-to-feed ratio of pigs fed adequate amount of Lys, which led to the conclusion that the reduced growth performance was due to the imbalance of AA rather than the antagonism (Southern and Baker, 1982). However, the Arg:Lys ratio in PNF was greater than that in diets used by Southern and Baker (1982). Further research is needed to evaluate the adequate ratio between Arg and Lys on digestibility of Lys. Values for the SID of CP and AA in broiler chickens fed PNF were comparable with the values reported by Iyayi and Adeola (2014). Zhang and Adeola (2016) reported that the N-corrected metabolizable energy in PNF determined by regression analysis was 4,112 kcal/kg DM. Based on the results of this experiment and previous studies, it was concluded that PNF can be a valuable

feed ingredient. However, further research is needed to determine the energy values for pigs as well as the effects of inclusion of PNF on growth performance of both broiler chickens and pigs.

In conclusion, there were no interactions for AA digestibility between species and ingredients, and the pattern of differences in AA digestibility among ingredients was similar between broiler chickens and pigs. Therefore, the relative quality of the AA among feed ingredients may be estimated by the information from other species. Further research is needed to compare AA digestibility between broiler chickens and pigs in using other feed ingredients.

4.6 Literature Cited

Adedokun, S. A., P. Jaynes, M. E. Abd El-Hack, R. L. Payne, and T. J. Applegate. 2014. Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. Poult. Sci. 93:420-428. doi:10.3382/ps.2013-03495

Adedokun, S. A., P. Utterback, C. M. Parsons, O. Adeola, M. S. Lilburn, and T. J. Applegate. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350-358. doi:10.1080/00071660902951321

Adeola, O., and D. E. King. 2006. Developmental changes in morphometry of the small intestine and jejunal sucrase activity during the first nine weeks of postnatal growth in pigs. J. Anim. Sci. 84:112-118. doi:10.2527/2006.841112x

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

American Oil Chemists' Society (AOCS). 2011. Official methods and recommended practices of the AOCS. 6th ed. American Oil Chemists' Society, Urbana, IL.

Araba, M., and N. M. Dale. 1990. Evaluation of protein solubility as an indicator of overprocessing soybean meal. Poult. Sci. 69:76-83. doi:10.3382/ps.0690076

Baker, K. M., B. G. Kim, and H. H. Stein. 2010. Amino acid digestibility in conventional, high-protein, or low-oligosaccharide varieties of full-fat soybeans and in soybean meal by weanling pigs. Anim. Feed Sci. Technol. 162:66-73. doi:10.1016/j.anifeedsci.2010.08.012

Batal, A., N. Dale, and M. Café. 2005. Nutrient composition of peanut meal. J. Appl. Poult. Res. 14:254-257. doi:10.1093/japr/14.2.254

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

D'Mello, J. P. F., and D. Lewis. 1970. Amino acid interactions in chick nutrition. 1. The interrelationship between lysine and arginine. Br. Poult. Sci. 11:299-311. doi:10.1080/00071667008415820

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634. doi:10.4141/cjas79-081

Goebel, K. P., and H. H. Stein. 2011. Ileal digestibility of amino acids in conventional and low-Kunitz soybean products fed to weanling pigs. Asian-Australas. J. Anim. Sci. 24:88-95. doi:10.5713/ajas.2011.90583

Heger, J., M. Wiltafsky, and J. Zelenka. 2016. Impact of different processing of full-fat soybeans on broiler performance. Czech J. Anim. Sci. 61:57-66. doi:10.17221/8728-CJAS

Herkelman, K. L., G. L. Cromwell, and T. S. Stahly. 1991. Effects of heating time and sodium metabisulfite on the nutritional value of full-fat soybeans for chicks. J. Anim. Sci. 69:4477-4486. doi:10.2527/1991.69114477x

Iyayi, E. A., and O. Adeola. 2014. Standardized ileal amino acid digestibility of feedstuffs in broiler chickens. Europ. Poult. Sci. 78. doi:10.1399/eps.2014.54

Kim, B. G., and T. Kim. 2010. A program for making completely balanced Latin Square designs employing a systemic method. Rev. Colomb. Cienc. Pecu. 23:277-282.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85(Suppl. 2):70-71. (Abstr.)

Kim, B. G., M. D. Lindemann, G. L. Cromwell, A. Balfagon, and J. H. Agudelo. 2007. The correlation between passage rate of digesta and dry matter digestibility in various stages of swine. Livest. Sci. 109:81-84. doi:10.1016/j.livsci.2007.01.082

Kong, C., and O. Adeola. 2013a. Comparative amino acid digestibility for broiler chickens and White Pekin ducks. Poult. Sci. 92:2367-2374. doi:10.3382/ps.2013-03042

Kong, C., and O. Adeola. 2013b. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Pekin ducks. Poult. Sci. 92:2381-2388. doi:10.3382/ps.2013-03179

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Kong, C., D. Ragland, and O. Adeola. 2014. Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in pigs. Asian-Australas. J. Anim. Sci. 27:1124-1130. doi:10.5713/ajas.2014.14232

Li, Q., X. Piao, J. Liu, Z. Zeng, S. Zhang, and X. Lei. 2014. Determination and prediction of the energy content and amino acid digestibility of peanut meals fed to growing pigs. Arch. Anim. Nutr. 68:196-210. doi:10.1080/1745039X.2014.910970

Liu, Y., N. W. Jaworski, O. J. Rojas, and H. H. Stein. 2016. Energy concentration and amino acid digestibility in high protein canola meal, conventional canola meal, and in soybean meal fed to growing pigs. Anim. Feed Sci. Technol. 212:52-62. doi:10.1016/j.anifeedsci.2015.11.017

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Palliyeguru, M. W. C. D., S. P. Rose, and A. M. Mackenzie. 2011. Effect of trypsin inhibitor activity in soya bean on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks. Br. Poult. Sci. 52:359-367. doi:10.1080/00071668.2011.577054

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186-192.

Park, C. S., A. R. Son, and B. G. Kim. 2014. Amino acid digestibility in oilseed meals fed to growing pigs. J. Anim. Sci. 92(Suppl. 2):649. (Abstr.)

Pond, W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2005. Basic animal nutrition and feeding. 5th ed. John Wiley and Sons, Inc., Hoboken, NJ.

Qin, G. X., M. W. A. Verstegen, and A. F. B. van der Poel. 1998. Effect of temperature and time during steam treatment on the protein quality of full-fat soybeans from different origins. J. Sci. Food Agric. 77:393-398. doi:10.1002/(SICI)1097-0010(199807)77:3<393::AID-JSFA59>3.0.CO;2-M

Ravindran, V., M. Abdollahi, and S. Bootwalla. 2014a. Nutrient analysis, apparent metabolisable energy and ileal amino acid digestibility of full fat soybean for broilers. Anim. Feed Sci. Technol. 197:233-240. doi:10.1016/j.anifeedsci.2014.08.014

Ravindran, V., M. R. Abdollahi, and S. M. Bootwalla. 2014b. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci. 93:2567-2577. doi:10.3382/ps.2014-04068

Rochell, S. J., T. J. Applegate, E. J. Kim, and W. A. Dozier III. 2012. Effects of diet type and ingredient composition on rate of passage and apparent ileal amino acid digestibility in broiler chicks. Poult. Sci. 91:1647-1653. doi:10.3382/ps.2012-02173

Sauvant, D., J. -M. Perez, and G. Tran. 2004. Tables of composition and nutritional value of feed materials. 2nd ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Sheppard, A. J., and T. S. Rudolf. 1991. Analysis of peanuts and peanut products for total lipids, fatty acids and proximates. Peanut Sci. 18:51-54. doi:10.3146/i0095-3679-18-1-14

Skrede, A., G. M. Berge, T. Storebakken, O. Herstad, K. G. Aarstad, and F. Sundstøl. 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Anim. Feed Sci. Technol. 76:103-116. doi:10.1016/S0377-8401(98)00208-9

Southern, L. L., and D. H. Baker. 1982. Performance and concentration of amino acids in plasma and urine of young pigs fed diets with excesses of either arginine or lysine. J. Anim. Sci. 55:857-866. doi:10.2527/jas1982.554857x

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742

Tahmasebi, S., and M. Toghyani. 2016. Effect of arginine and threonine administered in ovo on digestive organ developments and subsequent growth performance of broiler chickens. J. Anim. Physiol. Anim. Nutr. 100:947-956. doi:10.1111/jpn.12400

Valencia, D. G., M. P. Serrano, R. Lázaro, E. Jiménez-Moreno, and G. G. Mateos. 2009. Influence of micronization (fine grinding) of soya bean meal and full-fat soya bean on the ileal digestibility of amino acids for broilers. Anim. Feed Sci. Technol. 150:238-248. doi:10.1016/j.anifeedsci.2008.08.010

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Waldroup, P. W. 1982. Whole soybeans for poultry feeds. World's Poult. Sci. J. 38:28-35. doi:10.1017/WPS19820003

Walugembe, M., M. F. Rothschild, and M. E. Persia. 2014. Effects of high fiber ingredients on the performance, metabolizable energy and fiber digestibility of broiler and layer chicks. Anim. Feed Sci. Technol. 188:46-52. doi:10.1016/j.anifeedsci.2013.09.012

Woyengo, T. A., R. Jha, E. Beltranena, A. Pharazyn, and R. T. Zijlstra. 2014. Nutrient digestibility of lentil and regular- and low-oligosaccharide, micronized full-fat soybean fed to grower pigs. J. Anim. Sci. 92:229-237. doi:10.2527/jas.2013-6555

Xue, P. C., D. Ragland, and O. Adeola. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937-3944. doi:10.2527/jas.2014-7815

Zhang, F., and O. Adeola. 2016. Energy values of canola meal, cottonseed meal, bakery meal, and peanut flour meal for broiler chickens determined using the regression method. Poult. Sci. (in press). doi:10.3382/ps/pew239

Table 4.1 Analyzed chemical composition of full-fat soybean (FFSB), soybean meal containing 430 g/kg crude protein (SBM-43), soybean meal containing 470 g/kg crude protein (SBM-47), and peanut flour (PNF), g/kg as-fed basis

	Ingredient			
Item	FFSB	SBM-43	SBM-47	PNF
Dry matter	916	897	880	942
Gross energy, kcal/kg	5,239	4,247	4,223	5,708
Crude protein	358	433	467	466
AEE[1]	182.9	34.9	29.3	259.4
Crude fiber	53.0	58.4	35.6	26.4
Ash	46.8	55.7	54.3	33.0
Neutral detergent fiber	103.9	109.0	71.6	92.4
Acid detergent fiber	64.5	72.9	43.2	35.7
TIA, TIU/mg[2]	5.40	2.40	3.40	1.35
Urease activity, pH rise	0.02	0.06	0.02	< 0.02
Protein solubility, %[3]	89.90	82.08	78.65	78.42
Indispensable amino acid				
Arg	26.5	31.3	35.5	50.4
His	9.9	11.4	12.8	10.8
Ile	18.0	20.8	23.6	16.8
Leu	28.9	34.0	37.8	30.6
Lys	24.0	28.1	30.7	11.7
Met	5.1	6.0	6.6	5.0
Phe	19.0	22.5	24.9	24.1
Thr	14.4	17.0	18.1	11.7
Trp	4.4	5.9	6.5	4.7
Val	18.6	21.4	24.4	20.0
Dispensable amino acid				
Ala	16.0	19.0	20.8	17.9
Asp	41.6	48.9	54.4	52.2
Cys	5.2	5.8	6.6	5.1
Glu	62.8	77.1	86.6	85.5
Gly	17.0	19.2	20.7	27.1
Pro	17.7	21.0	23.1	18.2
Ser	16.3	19.1	20.1	18.2
Tyr	13.4	16.1	17.2	17.9

[1]Acid hydrolyzed ether extract.
[2]TIA = trypsin inhibitor activity; TIU = trypsin inhibitor unit.
[3]Measured by dissolving samples into 0.2% potassium hydroxide solution.

Table 4.2 Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[2]				
Item[1]	FFSB	SBM-43	SBM-47	PNF	NFD
Dextrose	510.5	535.5	567.5	598.0	640.0
Full-fat soybean	410.0	0.0	0.0	0.0	0.0
Soybean meal, 430 g/kg CP	0.0	350.0	0.0	0.0	0.0
Soybean meal, 470 g/kg CP	0.0	0.0	318.0	0.0	0.0
Peanut flour	0.0	0.0	0.0	314.0	0.0
Cornstarch	0.0	0.0	0.0	0.0	173.5
Solka-floc[3]	0.0	0.0	0.0	0.0	50.0
Soybean oil	15.0	50.0	50.0	15.0	50.0
Ground limestone	19.5	20.5	20.0	18.0	18.0
Monocalcium phosphate	11.0	10.0	10.5	21.0	21.0
Salt	4.0	4.0	4.0	4.0	0.0
Potassium carbonate	0.0	0.0	0.0	0.0	2.6
Magnesium oxide	0.0	0.0	0.0	0.0	2.0
Sodium bicarbonate	0.0	0.0	0.0	0.0	7.5
Choline chloride	0.0	0.0	0.0	0.0	2.5
Potassium chloride	0.0	0.0	0.0	0.0	2.9
Vitamin-mineral premix[4]	5.0	5.0	5.0	5.0	5.0
Chromic oxide premix[5]	25.0	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000	1,000

[1]CP = crude protein

[2]FFSB = full-fat soybean; SBM-43 = soybean meal containing 430 g/kg CP; SBM-47 = soybean meal containing 470 g/kg CP; PNF = peanut flour; NFD = N-free diet.

[3]International Fiber Corporation, North Tonawanda, NY.

[4]Provided the following quantities per kilogram 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.

[5]Provided 5 g chromic oxide per kilogram of complete diet.

Table 4.3 Analyzed concentration of dry matter, crude protein (CP), and amino acids (AA) in experimental diets, g/kg as-fed basis

	Diet[1]				
Item	FFSB	SBM-43	SBM-47	PNF	NFD
Dry matter	896	893	895	898	846
CP	149	155	146	152	8
Indispensable AA					
Arg	9.9	10.2	10.0	15.4	0.0
His	3.6	3.7	3.6	3.4	0.0
Ile	6.9	7.2	7.0	5.2	0.1
Leu	11.2	11.6	11.4	9.7	0.1
Lys	8.7	8.9	8.3	3.6	0.1
Met	1.9	1.9	1.8	1.4	0.0
Phe	7.3	7.6	7.5	7.3	0.0
Thr	5.5	5.7	5.5	3.7	0.0
Trp	1.6	2.0	1.7	1.0	0.0
Val	7.1	7.4	7.2	6.2	0.0
Dispensable AA					
Ala	6.2	6.5	6.3	5.7	0.0
Asp	16.3	16.9	16.4	16.8	0.1
Cys	1.9	1.9	1.8	1.5	0.0
Glu	25.4	26.8	26.1	28.1	0.2
Gly	6.3	6.5	6.2	8.5	0.0
Pro	6.9	7.2	7.1	5.8	0.0
Ser	6.1	6.5	6.3	5.9	0.0
Tyr	4.6	4.8	4.6	4.3	0.0

[1]FFSB = full-fat soybean; SBM-43 = soybean meal containing 430 g/kg CP; SBM-47 = soybean meal containing 470 g/kg CP; PNF = peanut flour; NFD = N-free diet.

Table 4.4 Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in full-fat soybean, soybean meals, and peanut flour for broiler chickens and pigs

	Broiler chickens				Pigs					*P*-value		
Item	FFSB	SBM-43	SBM-47	PNF	FFSB	SBM-43	SBM-47	PNF	RMSE	Diet	Species	Diet × species
CP	68.4^{b}	76.5^{a}	77.4^{a}	77.0^{a}	73.3^{y}	80.9^{x}	82.6^{x}	78.9^{xy}	4.76	< 0.001	0.002	0.781
Indispensable AA												
Arg	81.5^{b}	85.8^{ab}	87.5^{a}	90.1^{a}	86.2^{y}	91.2^{x}	92.0^{x}	93.2^{x}	2.87	< 0.001	< 0.001	0.731
His	74.1^{b}	80.6^{a}	82.2^{a}	79.9^{ab}	79.4^{z}	85.3^{xy}	87.5^{x}	82.7^{yz}	3.85	< 0.001	< 0.001	0.776
Ile	71.8^{b}	79.8^{a}	81.0^{a}	80.9^{a}	77.6^{y}	84.9^{x}	86.3^{x}	85.8^{x}	3.84	< 0.001	< 0.001	0.986
Leu	72.3^{b}	79.7^{a}	80.7^{a}	84.2^{a}	77.5^{z}	84.3^{y}	86.1^{xy}	89.3^{x}	3.76	< 0.001	< 0.001	0.993
Lys	73.4^{b}	79.1^{ab}	80.1^{a}	54.4^{c}	79.5^{y}	84.6^{xy}	86.3^{x}	60.5^{z}	3.97	< 0.001	< 0.001	0.993
Met	72.8^{b}	81.0^{a}	79.7^{a}	77.6^{ab}	79.6^{y}	87.3^{x}	88.3^{x}	87.3^{x}	3.97	< 0.001	< 0.001	0.600
Phe	76.2^{c}	81.7^{bc}	83.3^{ab}	87.9^{a}	79.4^{z}	85.3^{y}	86.9^{y}	91.5^{x}	3.47	< 0.001	< 0.001	0.998
Thr	62.8^{b}	71.5^{a}	72.4^{a}	67.9^{ab}	69.7^{y}	77.3^{x}	78.9^{x}	72.4^{xy}	5.17	< 0.001	< 0.001	0.925
Trp	74.6^{b}	85.1^{a}	84.2^{a}	79.3^{ab}	77.9^{y}	89.4^{x}	89.1^{x}	79.6^{y}	4.00	< 0.001	0.003	0.430
Val	68.5^{b}	77.5^{a}	78.6^{a}	81.3^{a}	73.6^{y}	81.9^{x}	83.9^{x}	85.5^{x}	4.39	< 0.001	< 0.001	0.987
Dispensable AA												
Ala	69.4^{b}	78.1^{a}	78.8^{a}	81.2^{a}	71.2^{y}	79.6^{x}	81.3^{x}	83.1^{x}	4.89	< 0.001	0.129	0.994
Asp	74.5^{b}	78.6^{ab}	80.8^{a}	79.0^{ab}	77.5^{z}	82.8^{xy}	84.8^{x}	78.8^{yz}	3.89	< 0.001	0.007	0.396
Cys	45.6^{b}	57.0^{ab}	47.1^{b}	66.5^{a}	63.9^{y}	75.6^{x}	77.3^{x}	71.3^{xy}	8.34	< 0.001	< 0.001	0.002
Glu	80.9^{b}	84.8^{ab}	86.8^{a}	86.3^{a}	82.3^{y}	87.8^{x}	88.9^{x}	87.5^{x}	3.48	< 0.001	0.035	0.886
Gly	64.1^{b}	73.1^{ab}	75.9^{a}	64.1^{b}	65.9^{yz}	74.8^{xy}	79.2^{x}	60.4^{z}	6.89	< 0.001	0.659	0.529
Pro	71.5^{b}	78.0^{ab}	80.0^{a}	81.8^{a}	75.0^{y}	81.1^{xy}	84.4^{x}	80.7^{xy}	4.82	< 0.001	0.051	0.422
Ser	69.7^{b}	78.3^{a}	79.0^{a}	76.1^{a}	74.1^{y}	82.1^{x}	84.1^{x}	76.5^{y}	3.94	< 0.001	0.002	0.385
Tyr	72.9^{b}	80.2^{a}	82.2^{a}	84.0^{a}	77.3^{y}	84.0^{x}	86.4^{x}	87.2^{x}	3.88	< 0.001	< 0.001	0.975

Note: FFSB = full-fat soybean; SBM-43 = soybean meal containing 430 g/kg CP; SBM-47 = soybean meal containing 470 g/kg CP; PNF = peanut flour.
Note: each least squares mean represents 8 observations except for PNF in pigs (7 observations).
$^{a\text{-}c;x\text{-}z}$Means within a row without a common superscript letter within species differ ($P < 0.05$).

Table 4.5 Basal ileal endogenous losses of crude protein (CP) and amino acids (AA) for broiler chickens and pigs, g/kg dry matter intake

Item	Broiler chickens	Pigs	SED	*P*-value
CP	10.6	11.0	2.03	0.847
Indispensable AA				
Arg	0.387	0.260	0.1075	0.245
His	0.176	0.121	0.0423	0.202
Ile	0.376	0.245	0.0884	0.153
Leu	0.595	0.392	0.1463	0.178
Lys	0.499	0.254	0.1498	0.121
Met	0.126	0.048	0.0396	0.072
Phe	0.349	0.232	0.0831	0.172
Thr	0.537	0.437	0.0973	0.309
Trp	0.064	0.040	0.0225	0.304
Val	0.472	0.330	0.1029	0.177
Dispensable AA				
Ala	0.395	0.354	0.0964	0.662
Asp	0.735	0.618	0.1662	0.475
Cys	0.223	0.122	0.0276	0.003
Glu	0.975	0.717	0.2323	0.271
Gly	0.421	0.746	0.1078	0.010
Pro	0.433	1.651	0.2939	0.007
Ser	0.452	0.356	0.0905	0.311
Tyr	0.256	0.196	0.0583	0.304

Note: each mean represents 8 observations for broiler chickens and 7 observations for pigs.

Table 4.6 Standardized ileal digestibility (%) of CP and AA in full-fat soybean, soybean meals, and peanut flour for broiler chickens and pigs

	Broiler chickens				Pigs					*P*-value		
Item	FFSB	SBM-43	SBM-47	PNF	FFSB	SBM-43	SBM-47	PNF	RMSE	Diet	Species	Diet × species
CP	74.4^{b}	82.3^{a}	83.5^{a}	82.8^{a}	79.5^{y}	86.9^{x}	89.0^{x}	85.0^{xy}	4.76	< 0.001	< 0.001	0.779
Indispensable AA												
Arg	84.8^{b}	89.0^{ab}	90.7^{a}	92.2^{a}	88.4^{y}	93.3^{x}	94.2^{x}	94.6^{x}	2.87	< 0.001	< 0.001	0.826
His	78.2^{b}	84.6^{a}	86.4^{a}	84.3^{ab}	82.2^{z}	88.1^{xy}	90.3^{x}	85.7^{yz}	3.85	< 0.001	0.002	0.758
Ile	76.4^{b}	84.2^{a}	85.5^{a}	87.0^{a}	80.6^{y}	87.7^{x}	89.3^{x}	89.7^{x}	3.84	< 0.001	< 0.001	0.958
Leu	76.8^{b}	84.1^{a}	85.2^{a}	89.4^{a}	80.5^{z}	87.2^{y}	89.0^{xy}	92.7^{x}	3.76	< 0.001	< 0.001	0.993
Lys	78.2^{b}	83.9^{ab}	85.2^{a}	66.1^{c}	81.9^{y}	87.0^{xy}	88.8^{x}	66.5^{z}	3.97	< 0.001	0.010	0.631
Met	78.4^{b}	86.6^{a}	85.6^{a}	85.2^{ab}	81.7^{y}	89.4^{x}	90.5^{x}	90.2^{x}	3.97	< 0.001	< 0.001	0.814
Phe	80.2^{c}	85.6^{bc}	87.2^{ab}	91.9^{a}	82.1^{z}	87.9^{y}	89.5^{y}	94.1^{x}	3.47	< 0.001	0.017	0.998
Thr	71.1^{b}	79.5^{a}	80.6^{a}	80.2^{a}	76.5^{y}	83.8^{x}	85.7^{x}	82.4^{xy}	5.17	< 0.001	0.002	0.844
Trp	78.0^{b}	87.8^{a}	87.4^{a}	84.8^{a}	80.0^{y}	91.1^{x}	91.1^{x}	83.0^{y}	4.00	< 0.001	0.081	0.265
Val	74.2^{b}	82.9^{a}	84.2^{a}	87.7^{a}	77.6^{y}	85.7^{x}	87.7^{x}	90.0^{x}	4.39	< 0.001	0.010	0.979
Dispensable AA												
Ala	74.7^{b}	83.2^{a}	84.1^{a}	87.1^{a}	76.0^{y}	84.2^{x}	86.1^{x}	88.3^{x}	4.89	< 0.001	0.281	0.994
Asp	78.3^{b}	82.3^{ab}	84.6^{a}	82.7^{ab}	80.7^{z}	85.9^{xy}	88.0^{x}	81.9^{yz}	3.89	< 0.001	0.032	0.397
Cys	55.5^{b}	67.0^{ab}	57.6^{b}	79.1^{a}	69.3^{y}	81.1^{x}	83.0^{x}	78.2^{x}	8.34	< 0.001	< 0.001	0.001
Glu	84.2^{b}	87.9^{ab}	89.9^{a}	89.2^{a}	84.7^{y}	90.1^{x}	91.2^{x}	89.7^{xy}	3.48	< 0.001	0.220	0.886
Gly	69.8^{bc}	78.6^{ab}	81.6^{a}	68.3^{c}	75.9^{yz}	84.5^{xy}	89.4^{x}	67.9^{z}	6.89	< 0.001	0.008	0.383
Pro	76.8^{b}	83.1^{ab}	85.2^{a}	88.2^{a}	95.2^{y}	100.5^{xy}	104.1^{x}	104.8^{x}	4.82	< 0.001	< 0.001	0.923
Ser	76.0^{b}	84.2^{a}	85.1^{a}	82.6^{a}	79.0^{y}	86.7^{x}	88.9^{x}	81.6^{y}	3.94	< 0.001	0.042	0.369
Tyr	77.6^{b}	84.7^{a}	86.9^{a}	89.0^{a}	80.9^{y}	87.4^{x}	90.0^{x}	91.1^{x}	3.88	< 0.001	0.007	0.971

Note: FFSB = full-fat soybean; SBM-43 = soybean meal containing 430 g/kg CP; SBM-47 = soybean meal containing 470 g/kg CP; PNF = peanut flour.
Note: each least squares mean represents 8 observations except for PNF in pigs (7 observations).
$^{a\text{-}c;x\text{-}z}$Means within a row without a common superscript letter within species differ ($P < 0.05$).

CHAPTER 5. DIGESTIBILITY OF AMINO ACID IN FULL-FAT CANOLA SEEDS, CANOLA MEAL, AND CANOLA EXPELLERS FED TO BROILER CHICKENS AND PIGS

A version of this chapter has been published in Journal of Animal Science.

Park, C. S., D. Ragland, A. Helmbrecht, J. K. Htoo, and O. Adeola. 2019. Digestibility of amino acid in full-fat canola seeds, canola meal, and canola expellers fed to broiler chickens and pigs. J. Anim. Sci. 97:803-812. doi:10.1093/jas/sky470

5.1 Abstract

Canola products including full-fat canola seeds (FFCS), canola meal (CM), and canola expellers (CE) have been used in diets for both broiler chickens and pigs. However, their ability to utilize the amino acids (AA) in canola products might be different from each other. Therefore, this study was conducted to compare the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and AA in broiler chickens and growing pigs fed FFCS, CM, and CE. Three diets were prepared to contain FFCS, CM, or CE as a sole source of nitrogen. A nitrogen-free diet was prepared. In Exp. 1, a total of 272 twenty-one-day-old male broiler chickens with an initial body weight (BW) of 932 ± 80.6 g were assigned to diets in a randomized complete block design with BW as a blocking factor. After 5 d of feeding, birds were euthanized by sodium pentobarbital, and ileal digesta samples were collected from distal two-third of the ileum. In Exp. 2, sixteen barrows were surgically fitted with T-cannulas at the distal ileum. After 8-d recovery period, pigs (initial BW = 18.9 ± 1.17 kg) were divided into 4 blocks based on BW and assigned to a quadruplicate 4 × 2 incomplete Latin Square design with 4 diets and 2 periods. Each period consisted of 5-d adaptation and 2-d ileal digesta collection periods. Data were analyzed as a 2 × 3 factorial treatment arrangement with effects of species (broiler chickens or pigs) and diets (FFCS, CM, or CE). There were interactions ($P < 0.05$) between species and experimental diets in the AID of all indispensable AA except for Lys. The AID of indispensable AA in FFCS for broiler chickens was greater ($P < 0.05$) than in pigs. Broiler chickens also had greater ($P < 0.05$) AID of Arg, His, Leu, Phe, and Val in CM compared with pigs; however, there were no differences in the AID of indispensable AA in CE between broiler chickens and pigs. The basal ileal endogenous losses of CP and AA, except Trp, in pigs were greater ($P < 0.05$) than in

broiler chickens. There were also interactions ($P < 0.05$) between species and experimental diets in the SID of all indispensable AA except for Lys. Broiler chickens fed the diet containing FFCS had greater ($P < 0.05$) SID of indispensable AA compared with pigs fed the same diet; however, the SID of indispensable AA in CM or CE were not different between broiler chickens and pigs. In conclusion, differences in digestibility of AA in canola products were affected by nonruminant animal species.
Key words: amino acid, canola meal, digestibility, full-fat canola seeds, poultry, swine

5.2 Introduction

Commercial diets for both broiler chickens and pigs have been formulated with similar feed ingredients due to ingredient commonality and similarity of gastrointestinal tract and digestive physiology. Moreover, experimental procedures to evaluate the digestibility of amino acids (**AA**) in feed ingredients are similar (Kong and Adeola, 2014). Differences in the digestion process, especially in the foregut, may affect the utilization of AA even though both broiler chickens and pigs have similar digestive physiology. In addition, physiochemical properties of feed ingredients may differently act on the utilization of AA in broiler chickens and pigs. Park et al. (2017) reported that the standardized ileal digestibility (**SID**) of most AA in full-fat soybean, hulled soybean meal (**SBM**), dehulled SBM, and peanut flour for pigs were greater than in broiler chickens but both species had differences in the SID of AA among test ingredients that were consistent between the species. However, it is unclear if the SID of AA for pigs is greater than that for broiler chickens in other feed ingredients and if the SID of AA in other feed ingredients shows similar pattern of differences within species.

Canola meal (**CM**), which is the co-product of solvent extraction of oil from full-fat canola seeds (**FFCS**), has been widely used in diets for both broiler chickens and pigs as a protein supplement, whereas, canola expellers (**CE**) is a co-product from double pressing using expeller for oil extraction from FFCS (Canola Council of Canada, 2015). In addition, FFCS has been also considered as an alternative feed ingredient for both broiler chickens and pigs due to its high energy and crude protein (**CP**) contents (Woyengo et al., 2014; Barekatain et al., 2015). However, digestibility of CP and AA in canola products may be influenced by processing procedure. Studies have been conducted to compare the digestibility of AA in canola products for broiler chickens (Woyengo et al., 2010; Adewole et al., 2017b) and for pigs (Maison and Stein, 2014; Adewole et

al., 2017a). However, most of previous studies have been used canola products originating from different crushing plants with a variety of canola seeds from different locations, but not from solvent- or expeller-extracted of the same canola seeds. Furthermore, the magnitude of influences from processing of canola products on digestibility of AA may be different between broiler chickens and pigs. Therefore, this study was conducted to test the hypothesis that the apparent ileal digestibility (**AID**) and SID of CP and AA in FFCS, CM, and CE, derived from solvent- or expeller-extracted of the same canola seeds, are not affected by canola seed processing in broiler chickens and pigs.

5.3 Materials and Methods

Experimental procedures using broiler chickens and pigs were reviewed and approved by the Purdue University Animal Care and Use Committee.

5.3.1 Ingredients and Experimental Diets

Full-fat canola seeds, CM, and CE originated from the same batch of canola seeds from University of Alberta (Edmonton, Alberta, Canada; Table 5.1). One batch of canola seeds was divided into 3 sub-batches. One sub-batch was used without oil removal as FFCS, one sub-batch had oil removed via expelling followed by solvent extraction to produce CM, and the last sub-batch had oil removed via expelling to produce CE. The FFCS was finely ground (< 0.75 mm) with dry ice using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany) before making the experimental diets. Three experimental diets containing FFCS, CM, or CE as a sole source of nitrogen (**N**) were prepared for both broiler chickens and pigs (Table 5.2). These diets were formulated to contain the same concentration of CP (N × 6.25) at 160 g/kg. Sucrose and cornstarch were added as energy sources. Soybean oil was added in the diet containing CM but not in the other diets due to the high concentration of acid hydrolyzed ether extract (**AEE**) in FFCS and CE. A N-free diet (**NFD**) was also prepared to determine the basal ileal endogenous losses (**BEL**) of CP and AA for broiler chickens and pigs. All diets were prepared to meet or exceed the estimated vitamin and mineral requirements for both broiler chickens and pigs in National Research Council (**NRC**; 1994, 2012). Chromic oxide at 5 g/kg was added in all diets as an indigestible marker.

5.3.2 Experiment 1: Digestibility of Crude Protein and Amino Acids for Broiler Chickens

Two-hundred-and-seventy-two male broiler chickens (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR) with a mean body weight (**BW**) of 42.5 g at d 0 post-hatching were obtained from a commercial hatchery. Birds were tagged and housed in electrically heated battery brooders (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR) with temperature at 35°C from d 0 to 7, at 31°C from d 7 to 14, and at 27°C from d 14 to 25. Before the experimental period, birds were fed a corn-SBM-based standard starter diet (210 g CP/kg) for 20 d. On d 21, individual BW of birds were obtained (mean initial BW = 932 ± 80.6 g), and then birds were allotted to 4 dietary treatments in a randomized complete block design with BW as a blocking factor using a spreadsheet program (Kim and Lindemann, 2007). Eight replicate cages were assigned to each diet with 8 birds per cage except for the NFD treatment, which had 10 birds per cage. Birds had ad libitum access to feed and water during the experimental period. After feeding experimental diets for 5 d, birds were euthanized by the injection of sodium pentobarbital (156 mg/kg BW; Fatal-Plus; Vortech Pharmaceuticals, Ltd., Dearborn, MI). Collection of ileal digesta and sample processing were as previously described by Park et al. (2017).

5.3.3 Experiment 2: Digestibility of Crude Protein and Amino Acids for Pigs

Sixteen barrows were surgically fitted with T-cannulas at the distal ileum by following the procedure by Dilger et al. (2004). Pigs were moved into individual metabolism crates (1.22 × 1.22 m) equipped with a feeder and a nipple drinker and received a corn-SBM-based standard grower diet (194 g CP/kg) for 8 d of recovery period. After the recovery period, pigs with an initial BW of 18.9 ± 1.17 kg were divided into 4 blocks based on BW and allotted to a quadruplicate 4 × 2 incomplete Latin Square design with 4 experimental diets and 2 periods using a spreadsheet program (Kim and Kim, 2010).

Feeding, collection of ileal digesta, and sample processing were conducted by following the procedures described by Park et al. (2017).

5.3.4 Chemical Analysis

Ileal digesta samples from broiler chickens and pigs were freeze-dried before further analyses. Test ingredients, experimental diets, and ileal digesta samples were finely ground (< 0.75

mm) using a centrifugal grinder and analyzed for dry matter (**DM**) by drying at 105°C for 24 h in a forced-air drying oven [Precision Scientific Co., Chicago, IL; method 934.01; Association of Official Analytical Chemists (**AOAC)**, 2006]. Nitrogen concentration in test ingredients, experimental diets, and ileal digesta samples were analyzed by combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP concentration was obtained by multiplying the N concentration by 6.25. The concentration of AA in test ingredients, experimental diets, and ileal digesta samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Briefly, test ingredients, diets, and ileal digesta samples were hydrolyzed in 6 M HCl (or BaOH for the analysis of Trp) at 110°C for 24 h under N atmosphere. For Met and Cys analysis, samples were oxidized by performic acid before acid hydrolysis. Hydrolyzed samples were analyzed for AA concentration by high-performance liquid chromatography after post-column derivatization [method 982.30 E (a, b, c); AOAC, 2006]. Test ingredients were analyzed for gross energy using an isoperibol bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) and ash (method 942.05; AOAC, 2006). The concentration of AEE (method 954.02; AOAC, 2006) were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Test ingredients were also analyzed for crude fiber (method 978.10; AOAC, 2006), neutral detergent fiber (**NDF**; Van Soest et al., 1991), and acid detergent fiber [**ADF**; method 973.18 (AD); AOAC, 2006] using a fiber analyzer (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY) after extraction of fat for 12 h in the analyses of NDF and ADF and for 24 h in the analysis of crude fiber. Experimental diets and ileal digesta samples were analyzed for the concentrations of Cr using a spectrophotometer (Spark 10M; Tecan Group Ltd., Männedorf, Switzerland) at 450 nm of absorption after wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

5.3.5 Calculations and Statistical Analysis

Calculations for the AID and SID of CP and AA as well as the BEL of CP and AA for broiler chickens and pigs were as previously described by Park et al. (2017).

Data from Exp. 1 and 2 were pooled and considered a 2 × 3 factorial arrangement with effects of species (broiler chickens or pigs) and experimental diets (FFCS, CM, or CE). Before statistical analysis, outlier was tested using 2.5 times interquartile range, and one pig fed NFD in period 1 of Exp. 2 was removed as outlier. Data were analyzed by ANOVA using general linear

model procedure of SAS (SAS Inst. Inc., Cary, NC). Independent variables in the model were species, experimental diets, interaction between species and experimental diets, and block within species. In order to match the blocking factors of Exp. 2 with Exp. 1, the components of Latin square design were rearranged. The experimental design of Exp. 2 was a quadruplicate 4 × 2 incomplete Latin Square design with 4 blocks of BW (i.e., 4 pigs per block), 4 columns of 4 pigs on 4 diets in each of 4 blocks, and 2 periods in each of 4 blocks. The 4 columns of 4 pigs on 4 diets were merged into diets to remove column effects from the statistical model, and 2 periods were multiplied with 4 blocks to generate 8 blocks. With this, the effects of the 4 blocks in each of the 2 periods are unrelated. Block effects in Exp. 2 were consistent with those in Exp. 1, but were considered separately in the model (i.e., block nested within species as independent variable). Least squares means were separated by pairwise comparison with the Tukey's adjustment if there was an interaction. Data for the BEL of CP and AA were analyzed by two-sample, two-tailed *t* tests to compare values between broiler chickens and pigs. In all statistical analyses, the experimental unit was cage for Exp. 1 and pig for Exp. 2, and the significance was determined at $P < 0.05$.

5.4 Results

In Exp. 1, one bird receiving the diet containing FFCS was removed from the experiment because of abnormal health condition. All other birds were in good condition during the experimental period. In Exp. 2, all pigs were in good condition during the experimental period.

5.4.1 Chemical Analysis

The analyzed concentration of CP in test ingredients ranged from 248 g/kg in FFCS to 436 g/kg in CM on an as-fed basis (Table 5.1). The AEE concentration in FFCS was 339 g/kg as-fed basis, but it was 24 g/kg as-fed basis in CM. The analyzed concentration of CP and AA in experimental diets were close to the values calculated by the analyzed concentration of CP and AA in test ingredients (Table 5.3).

5.4.2 Apparent Ileal Digestibility of Crude Protein and Amino Acids

There were interactions ($P < 0.05$) between experimental diets and species in the AID of AA except Lys, Gly, Pro, and Ser (Table 5.4). The AID of indispensable AA in FFCS for broiler chickens were greater ($P < 0.05$) than for pigs. Broiler chickens also had greater ($P < 0.05$) AID of Arg, His, Leu, Phe, and Val in CM compared with pigs; however, there were no differences in AID of indispensable AA in CE between broiler chickens and pigs. The AID of CP and Lys for broiler chickens was greater ($P < 0.001$) than for pigs regardless of the experimental diets. Broiler chickens fed FFCS or CM had greater ($P < 0.05$) AID of Ala, Asp, Glu, and Tyr compared with pigs. The AID of Ala in CE for broiler chickens was greater ($P < 0.05$) than for pigs. The AID of Gly, Pro, and Ser for broiler chickens were greater ($P < 0.001$) than for pigs regardless of the experimental diets.

5.4.3 Standardized Ileal Digestibility of Crude Protein and Amino Acids

The BEL of CP and AA, except Trp, in pigs were greater ($P < 0.05$) than in broiler chickens (Table 5.5). The BEL of indispensable AA ranged from 44 mg/kg DM intake (**DMI**) for Trp to 446 mg/kg DMI for Thr in broiler chickens and from 58 mg/kg DMI for Trp to 703 mg/kg DMI for Leu in pigs.

Similar to the results for AID values, interactions ($P < 0.05$) between experimental diets and species were observed for the SID of AA except Lys, Gly, Pro, and Ser (Table 5.6). Broiler chickens fed the diet containing FFCS had greater ($P < 0.05$) SID of indispensable AA compared with pigs fed the same diet; however, the SID of indispensable AA in CM or CE were not different between broiler chickens and pigs. Within broiler chickens, the SID of all indispensable AA, except Trp, was not different from values calculated for CM and CE. However, within pigs, the SID of all indispensable AA, except Trp, in FFCS was less ($P < 0.05$) than in CE, but the SID of all indispensable AA, except Met, in FFCS was not different from values obtained for CM. The SID of CP and Lys for broiler chickens was greater ($P < 0.001$) than for pigs regardless of experimental diet. The SID of Ala, Asp, Cys, Glu, and Tyr in FFCS fed to broiler chickens was greater ($P < 0.05$) than for pigs; however, values in CM or CE for broiler chickens were not different from pigs.

5.5 Discussion

The analyzed concentration of CP in FFCS was similar to values reported by González-Vega and Stein (2012) and NRC (2012) but was greater than values in other reports (Sauvant et al., 2004; Seneviratne et al., 2011; Barekatain et al., 2015; Eklund et al., 2015). In addition, the analyzed concentration of CP in CM was also greater than values by Sauvant et al. (2004), NRC (2012), Maison and Stein (2014), and Adewole et al. (2016, 2017a). These differences may be due to the low concentration of AEE in the FFCS used in this experiment. The analyzed AEE concentration in FFCS was less than previously reported values (González-Vega and Stein, 2012; NRC, 2012; Barekatain et al., 2015). Barthet and Daun (2011) reported that the general oil content in canola seeds is usually greater than 440 g/kg and there is a negative correlation between protein and oil contents in canola seeds, which agreed with the high CP and low AEE concentrations in FFCS used in this study. Therefore, the CP in FFCS might be concentrated when producing CM which led to increased CP concentration in CM used in this study. This is also in accordance with the low concentration of AEE in CM compared with values reported by Maison and Stein (2014). However, the concentration of CP in CE was within the range of values in previous studies (NRC, 2012; Maison and Stein, 2014; Woyengo et al., 2016). This may be due to the greater concentration of AEE in CE that remains after oil extraction. The analyzed concentration of AEE in CE was greater than the range of values reported by Maison and Stein (2014).

Although the low concentration of AEE in FFCS used in this experiment is partly explained by the greater concentration of CP in both FFCS and CM, it was considerably less than the standard value at minimum 440 g/kg suggested in Barthet and Daun (2011). This may be due to the loss of oil during grinding. The concentration of NDF and ADF in FFCS were in agreement with values in previous studies (Sauvant et al., 2004; González-Vega and Stein, 2012; NRC, 2012; Barekatain et al., 2015). However, the concentration of NDF and ADF in CM were less than previously reported values (Sauvant et al., 2004; NRC, 2012; Li et al., 2015; Adewole et al., 2017a), and the concentration of NDF and ADF in CE were also less than values reported by NRC (2012) and Woyengo et al. (2016). This may be due to the differences in oil extraction process among studies. It may be speculated that there was a loss of cell wall components during the oil extraction process for CM and CE used in the current study, which may also explain the fact that the concentration of NDF and ADF in FFCS was similar to values in CE.

The BEL of CP and AA, except Trp, in pigs were greater than in broiler chickens, which is not consistent with the results reported in Park et al. (2017). However, the BEL of CP and AA agreed with the values reported in previous studies for broiler chickens (Kong and Adeola, 2013; Toghyani et al., 2015; Park et al., 2017) and for pigs (Park et al., 2013; Maison and Stein, 2014; Wang et al., 2018). The reason for the greater BEL of CP and AA in pigs compared with broiler chickens may be due to the shorter absolute length of small intestine in broiler chickens compared with pigs. Although the length of small intestine relative to BW in broiler chickens is longer than in pigs (Park et al., 2017), pigs have greater physical area to lose the intestinal cells compared with broiler chickens. Nyachoti et al. (1997) reported that majority of the BEL of N in pigs is contributed from tissues of the small intestine. Therefore, the BEL of CP and AA in broiler chickens may contain less sloughed cells or mucin proteins compared with pigs.

Interactions between experimental diets and species were observed in both AID and SID of AA except Lys, Gly, Pro, and Ser. These observations were mainly due to the greater digestibility of AA in FFCS for broiler chickens compared with pigs. However, Park et al. (2017) reported that there were no interactions for digestibility of CP and most AA between broiler chickens and pigs fed semi-purified diets containing full-fat soybean, SBM, and peanut flour and that the digestibility of CP and AA for pigs was greater than for broiler chickens. The reason for this discrepancy remains unclear; however, it may be due to the altered digestive functions in foregut of broiler chickens from increased intake of dietary fiber from FFCS. Birds fed diets containing 30 g/kg sugar beet pulp or oat hulls as a source of soluble or insoluble fiber, respectively, had increased weight of gizzard relative to BW and decreased pH of the digesta in proventriculus and gizzard compared with birds fed the control diet and the diet containing 30 g/kg cellulose (Jiménez-Moreno et al., 2009). Also, Jiménez-Moreno et al. (2010) found similar observations using finely ground (< 0.2 mm) sugar beet pulp or oat hulls at 30 g/kg of the diet. It is unclear whether fiber components and structure in FFCS has a beneficial effect on gizzard similar to sugar beet pulp or oat hulls and whether this beneficial effect appears after short-term feeding (i.e., 5 d) of the diet containing FFCS. However, it may be speculated that the digestive function of gizzard improved the digestibility of AA in FFCS, which is not applicable for pigs due to the absence of gizzard. On the other hand, increased intake of fiber in pigs fed the diet containing FFSC might reduce digestibility of AA. Fan et al. (1996) reported negative correlations between the concentration of NDF and the AID of CP and AA, except Arg, in pigs fed diets containing 6 CM

originating from different processing plants. Compared with the results reported in Park et al. (2017) where all the experimental diets were prepared as semi-purified diets based on dextrose, the diet containing FFCS used in the current experiment was prepared to contain 720 g/kg FFCS, which resulted in increased consumption of fiber to animals compared with other diets in the current experiment as well as the diets used by Park et al. (2017). Therefore, the effect of the concentration of fiber in the diet containing FFCS may have resulted in the interactions between species (broiler chickens or pigs) and diets (FFCS, CM, or CE). Further research is needed to verify the effects of fiber contents in canola products on traits of digestive organs in broiler chickens.

There were no differences in the AID and SID of CP and most AA among FFCS, CM, and CE fed to broiler chickens. The observation is in agreement with observations by Lee et al. (1995) who reported that the true digestibility of most AA in FFCS was not different from values for CM if measured using the force-fed rooster assay. Woyengo et al. (2010) also reported that the SID of CP and indispensable AA, except Thr, in CM were not different from those in CE. The SID of AA in full-fat rapeseed reported by Szczurek (2010) were less than the SID of AA in FFCS observed in the current study, which may be due to differences in variety of seeds. The SID of CP and indispensable AA in CM for broiler chickens obtained in this experiment was in agreement with reported values (Woyengo et al., 2010; Adewole et al., 2017b; Rad-Spice et al., 2018). The SID of CP and indispensable AA in CE obtained in this experiment was also comparable to values reported in previous studies (Woyengo et al., 2010; Bryan et al., 2017).

In pigs, however, the SID of most AA in FFCS were less than in CE, which were not different from CM. Similar with the possible reason for the interactions, it may be speculated that increased intake of fiber in pigs fed the diet containing FFCS negatively affected the digestibility of AA in FFCS. However, it remains unclear why the SID of CP and most AA in FFCS were not different from those in CM. González-Vega and Stein (2012) reported that the SID of CP, Arg, His, Lys, and Trp in FFCS were not different from those in CM, but the SID of the remaining indispensable AA in FFCS were less than in CM. This discrepancy may be due to the differences in sources of FFCS used in the studies. In addition, the differences for FFCS among studies may also be due to differences in grinding procedures. In the current experiment, the same batch of FFCS was used to produce CM; however, in González-Vega and Stein (2012), FFCS and CM were obtained from different plants. Moreover, the SID of CP and AA in FFCS observed in the current study were also greater than values reported in González-Vega and Stein (2012), which may be

also due to the differences in sources of FFCS used in studies. The SID of AA, except Trp, in FFCS were in agreement with values reported by NRC (2012).

In the current experiment for pigs, the SID of CP and AA in CM were not different from those in CE. However, Maison and Stein (2014) reported that the mean values for the SID of CP and most AA in 10 samples of 00-rapeseed meal were less than mean value for 5 samples of 00-rapeseed expellers. Woyengo et al. (2016) also reported that the SID of CP and most AA in CM were less than in CE in which both CM and CE originated from the same genus but different plants. Maison and Stein (2014) and Woyengo et al. (2016) suggested that lower SID of AA in CM than CE might be due to the heat damage of CM during desolventizing process. Therefore, it may be speculated that CM used in the current experiment were not damaged by heat during oil extraction process. Thus, the oil extraction process did not affect the SID of CP and AA in canola co-products that originated from the same batch of FFCS used in the current study. In addition, soybean oil was added to the diet containing CM but not in the diet containing FFCS and CE to prevent the potential effect of the dietary concentration of oil on digestibility of CP and AA in the current experiment. The SID of indispensable AA in CM observed in the current experiment were within the range of the previously reported values (NRC, 2012; Maison and Stein, 2014; Li et al., 2015; Woyengo et al., 2016; Adewole et al., 2017a). The SID of indispensable AA in CE were also comparable with the values reported in the previous studies (NRC, 2012; Woyengo et al., 2016) and the values reported by Maison and Stein (2014) despite of greater concentration of AEE. Kil and Stein (2011) reported that pigs fed diets containing 50 g/kg soybean oil or choice white grease had greater AID of Arg, Leu, and Val than pigs fed control diet possibly due to the increased retention time of digesta in gastrointestinal tract due to fat addition. However, the concentration of AEE in CE used in the current study was 44% greater than mean value (115.2 g/kg) of 5 CE used by Maison and Stein (2014). Therefore, it may be concluded that the greater concentration of AEE in CE used in the current study was not enough to increase the digestibility of AA in CE.

In conclusion, there were interactions between canola products and species. The SID of AA, except Lys, Gly, Pro, and Ser, in FFCS for broiler chickens was greater than for pigs. In broiler chickens, there were no differences in the SID of CP and indispensable AA, except Trp, among FFCS, CM, and CE. However, in pigs, the SID of all indispensable AA, except Lys and Trp, in FFCS was less than CE, but not different from those in CM.

5.6 Literature Cited

Adewole, D. I., A. Rogiewicz, B. Dyck, C. M. Nyachoti, and B. A. Slominski. 2017a. Standardized ileal digestible amino acid contents of canola meal from Canadian crushing plants for growing pigs. J. Anim. Sci. 95:2670-2679. doi:10.2527/jas2017.1372

Adewole, D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2016. Chemical and nutritive characteristics of canola meal from Canadian processing facilities. Anim. Feed Sci. Technol. 222:17-30. doi:10.1016/j.anifeedsci.2016.09.012

Adewole, D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2017b. Effects of canola meal source on the standardized ileal digestible amino acids and apparent metabolizable energy contents for broiler chickens. Poult. Sci. 96:4298-4306. doi:10.3382/ps/pex245

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Barekatain, M. R., S. B. Wu, M. Toghyani, and R. A. Swick. 2015. Effects of grinding and pelleting condition on efficiency of full-fat canola seed for replacing supplemental oil in broiler chicken diets. Anim. Feed Sci. Technol. 207:140-149. doi:10.1016/j.anifeedsci.2015.05.020

Barthet, V. J., and J. K. Daun. 2011. Seed morphology, composition, and quality. In: J. K. Daun, N. A. M. Eskin, and D. Hickling, editors, Canola: Chemistry, production, processing, and utilization. AOCS Press, Urbana, IL. p. 119-162.

Bryan, D. D. S. L., J. L. MacIsaac, B. M. Rathgeber, N. L. McLean, and D. M. Anderson. 2018. Meal residual oil level and heat treatment after oil extraction affects the nutritive value of expeller-pressed canola meal for broiler chickens. Can. J. Anim. Sci. 97:658-667. doi:10.1139/cjas-2015-0207

Canola Council of Canada. 2015. Canola meal feeding guide. 5th rev. ed. Canola Council of Canada, Winnipeg, Manitoba, Canada.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

Eklund, M., N. Sauer, F. Schöne, U. Messerschmidt, P. Rosenfelder, J. K. Htoo, and R. Mosenthin. 2015. Effect of processing of rapeseed under defined conditions in a pilot plant on chemical composition and standardized ileal amino acid digestibility in rapeseed meal for pigs. J. Anim. Sci. 93:2813-2825. doi:10.2527/jas2014-8210

Fan. M. Z., W. C. Sauer., and V. M. Gabert. 1996. Variability of apparent ileal amino acid digestibility in canola meal for growing-finishing pigs. Can. J. Anim. Sci. 76:563-569. doi:10.4141/cjas96-084

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634. doi:10.4141/cjas79-081

González-Vega. J. C., and H. H. Stein. 2012. Amino acid digestibility in canola, cottonseed, and sunflower products fed to finishing pigs. J. Anim. Sci. 90:4391-4400. doi:10.2527/jas2011-4631

Jiménez-Moreno, E., J. M. González-Alvarado, A. de Coca-Sinova, R. Lázaro, and G. G. Mateos. 2009. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. Anim. Feed Sci. Technol. 154:93-101. doi:10.1016/j.anifeedsci.2009.06.020

Jiménez-Moreno, E., J. M. González-Alvarado, D. González-Sánchez, R. Lázaro, and G. G. Mateos. 2010. Effects of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age. Poult. Sci. 89:2197-2212. doi:10.3382/ps.2010-00771

Kil, D. Y, and H. H. Stein. 2011. Dietary soybean oil and choice white grease improve apparent ileal digestibility of amino acids in swine diets containing corn, soybean meal, and distillers dried grains with solubles. Rev. Colomb. Cienc. Pecu. 24:248-253.

Kim, B. G., and T. Kim. 2010. A program for making completely balanced Latin Square designs employing a systemic method. Rev. Colomb. Cienc. Pecu. 23:277-282.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85(Suppl. 2):70-71. (Abstr.)

Kong, C., and O. Adeola. 2013. Comparative amino acid digestibility for broiler chickens and White Pekin ducks. Poult. Sci. 92:2367-2374. doi:10.3382/ps.2013-03042

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Lee, K. H., G. H. Qi, and J. S. Sim. 1995. Metabolizable energy and amino acid availability of full-fat seeds, meals, and oils of flax and canola. Poult. Sci. 74:1341-1348. doi:10.3382/ps.0741341

Li, P., F. Wang, F. Wu, J. Wang, L. Liu, and C. Lai. 2015. Chemical composition, energy and amino acid digestibility in double-low rapeseed meal fed to growing pigs. J. Anim. Sci. Biotechnol. 6:37. doi:10.1186/s40104-015-0033-0

Maison. T., and H. H. Stein. 2014. Digestibility by growing pigs of amino acids in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe. J. Anim. Sci. 92:3502-3514. doi:10.2527/jas2014-7748

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149-163. doi:10.4141/A96-044

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110-3119. doi:10.2527/jas2017.1404

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186-192.

Rad-Spice, M., A. Rogiewicz, J. Jankowski, and B. A. Slominski. 2018. Yellow-seeded B. *napus* and B. *juncea* canola. Part 1. Nutritive value of the meal for broiler chickens. Anim. Feed Sci. Technol. 240:66-77. doi:10.1016/j.anifeedsci.2018.03.017

Sauvant, D., J.-M. Perez, and G. Tran. 2004. Tables of composition and nutritional value of feed materials. 2nd ed. Wageningen Acad. Publ., Wageningen, the Netherlands.

Seneviratne, R. W., E. Beltranena, R. W. Newkirk, L. A. Goonewardene, and R. T. Zijlstra. 2011. Processing conditions affect nutrient digestibility of cold-pressed canola cake for grower pigs. J. Anim. Sci. 89:2452-2461. doi:10.2527/jas.2010-3569

Szczurek, W. 2010. Standardized ileal digestibility of amino acids in some cereals, rapeseed products and maize DDGS for broiler chickens at the age of 14 days. J. Anim. Feed Sci. 19:72-80. doi:10.22358/jafs/66271/2010

Toghyani, M., N. Rodgers, P. A. Iji, and R. A. Swick. 2015. Standardized ileal amino acid digestibility of expeller-extracted canola meal subjected to different processing conditions for starter and grower broiler chickens. Poult. Sci. 94:992-1002. doi:10.3382/ps/pev047

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Wang, T., S. O. Osho, and O. Adeola. 2018. 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. J. Anim. Sci. 96:4731-4742. doi:10.1093/jas/sky326

Woyengo, T. A., E. Beltranena, and R. T. Zijlstra. 2014. NONRUMINANT NUTRITION SYMPOSIUM: Controlling feed cost by including alternative ingredients into pig diets: A review. J. Anim. Sci. 92:1293-1305. doi:10.2527/jas2013-7169

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci. 89:1182-1189. doi:10.3382/ps.2009-00595

Woyengo, T. A., J. E. Sánchez, J. Yáñez, E. Beltranena, M. Cervantes, A. Morales, and R. T. Zijlstra. 2016. Nutrient digestibility of canola co-products for grower pigs. Anim. Feed Sci. Technol. 222:7-16. doi:10.1016/j.anifeedsci.2016.09.009

Table 5.1 Analyzed nutrient composition of full-fat canola seeds (FFCS), canola meal (CM), and canola expellers (CE), g/kg as-fed basis

	Ingredient		
Item	FFCS	CM	CE
Dry matter	949	905	926
Gross energy, kcal/kg	6,378	4,415	5,141
Crude protein (nitrogen × 6.25)	248	436	365
Acid hydrolyzed ether extract	339	24	166
Crude fiber	73.3	104.0	88.1
Ash	39.4	63.5	53.1
Neutral detergent fiber	194	213	179
Acid detergent fiber	155	180	150
Indispensable amino acids			
Arg	14.6	25.0	21.0
His	6.4	11.0	9.2
Ile	10.0	16.7	14.1
Leu	17.0	28.9	24.3
Lys	14.0	24.3	20.6
Met	4.8	7.9	6.7
Phe	10.0	16.8	14.2
Thr	9.9	16.9	14.3
Trp	2.7	4.9	4.0
Val	12.3	21.3	18.3
Dispensable amino acids			
Ala	10.4	18.4	15.3
Asp	17.1	28.2	23.9
Cys	5.8	10.2	8.6
Glu	40.3	72.0	59.5
Gly	12.0	20.3	17.0
Pro	9.2	15.8	13.5
Ser	12.9	23.4	18.6
Tyr	6.7	10.9	9.5

Table 5.2 Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[1]			
Item	FFCS	CM	CE	NFD
Sucrose	121.0	390.5	350.0	500.0
Cornstarch	100.0	100.0	100.0	316.0
Full-fat canola seeds	720.0	0.0	0.0	0.0
Canola meal	0.0	421.0	0.0	0.0
Canola expellers	0.0	0.0	494.0	0.0
Soybean oil	0.0	30.0	0.0	50.0
Cellulose[2]	0.0	0.0	0.0	50.0
Ground limestone	10.0	8.5	8.0	13.0
Monocalcium phosphate	15.0	16.0	14.0	23.5
Salt	4.0	4.0	4.0	0.0
Potassium carbonate	0.0	0.0	0.0	2.6
Magnesium oxide	0.0	0.0	0.0	2.0
Sodium bicarbonate	0.0	0.0	0.0	7.5
Choline chloride	0.0	0.0	0.0	2.5
Potassium chloride	0.0	0.0	0.0	2.9
Vitamin-mineral premix[3]	5.0	5.0	5.0	5.0
Chromic oxide premix[4]	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000

[1]FFCS = full-fat canola seeds; CM = canola meal; CE = canola expellers; NFD = nitrogen-free diet.
[2]Solka-Floc 40 FCC, International Fiber Corporation, North Tonawanda, NY.
[3]Provided the following quantities per kilogram 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.
[4]Provided 5 g chromic oxide per kilogram of complete diet.

Table 5.3 Analyzed concentration of dry matter, crude protein (CP), and amino acids (AA) in experimental diets, g/kg as-fed basis

	Diet[1]			
Item	FFCS	CM	CE	NFD
Dry matter	949	951	953	960
CP (nitrogen × 6.25)	178	171	179	6.43
Indispensable AA				
Arg	10.4	10.1	10.2	0.0
His	4.6	4.5	4.6	0.0
Ile	7.1	7.0	7.1	0.1
Leu	12.2	12.0	12.2	0.2
Lys	10.1	10.1	10.3	0.1
Met	3.2	3.1	3.2	0.0
Phe	7.0	7.0	7.1	0.1
Thr	7.0	7.0	7.0	0.0
Trp	2.8	2.2	2.1	0.0
Val	8.9	8.8	8.8	0.1
Dispensable AA				
Ala	7.6	7.6	7.7	0.1
Asp	12.3	11.7	11.9	0.2
Cys	4.1	4.2	4.2	0.0
Glu	31.2	30.1	30.1	0.2
Gly	8.6	8.4	8.5	0.1
Pro	6.7	6.6	6.5	0.1
Ser	9.0	9.4	9.8	0.0
Tyr	4.5	4.2	4.4	0.1

[1]FFCS = full-fat canola seeds; CM = canola meal; CE = canola expellers; NFD = nitrogen-free diet.

Table 5.4 Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in full-fat canola seeds (FFCS), canola meal (CM), and canola expellers (CE) for broiler chickens and pigs

	Broiler chickens			Pigs				*P*-value		
Item	FFCS	CM	CE	FFCS	CM	CE	SD	Diet	Species	Diet × species
CP	75.9	73.3	75.1	65.7	66.4	71.0	3.46	0.037	< 0.001	0.055
Indispensable AA										
Arg	87.3^{a}	85.5^{a}	86.5^{a}	78.7^{c}	80.0^{bc}	83.4^{ab}	2.85	0.077	< 0.001	0.035
His	85.5^{a}	82.9^{a}	84.3^{a}	76.8^{c}	78.8^{bc}	82.0^{ab}	2.66	0.043	< 0.001	0.006
Ile	78.3^{a}	75.8^{ab}	76.6^{a}	68.5^{c}	70.8^{bc}	75.2^{ab}	3.40	0.062	< 0.001	0.006
Leu	81.9^{a}	79.4^{a}	80.3^{a}	71.0^{c}	72.8^{bc}	77.6^{ab}	3.37	0.051	< 0.001	0.007
Lys	82.0	79.6	80.8	70.5	72.2	74.6	3.24	0.278	< 0.001	0.064
Met	86.2^{a}	85.0^{ab}	85.9^{a}	78.1^{c}	81.9^{b}	84.6^{ab}	2.32	0.003	< 0.001	< 0.001
Phe	81.0^{a}	79.7^{a}	80.4^{a}	69.9^{c}	73.1^{bc}	77.4^{ab}	3.38	0.021	< 0.001	0.008
Thr	72.4^{a}	69.3^{ab}	70.7^{ab}	62.8^{c}	64.5^{bc}	69.5^{ab}	4.14	0.095	< 0.001	0.026
Trp	94.9^{a}	85.5^{b}	87.9^{b}	88.0^{b}	83.9^{b}	86.7^{b}	2.94	< 0.001	< 0.001	0.018
Val	75.1^{a}	72.6^{a}	73.1^{a}	64.4^{c}	66.2^{bc}	71.3^{ab}	3.93	0.105	< 0.001	0.013
Dispensable AA										
Ala	81.4^{a}	79.2^{ab}	79.9^{a}	69.4^{d}	71.9^{cd}	75.2^{bc}	2.91	0.083	< 0.001	0.004
Asp	79.0^{a}	76.1^{ab}	77.5^{ab}	67.2^{c}	68.1^{c}	72.5^{bc}	3.74	0.098	< 0.001	0.048
Cys	80.0^{a}	74.8^{bc}	76.2^{ab}	70.9^{c}	70.9^{c}	74.3^{bc}	3.38	0.071	< 0.001	0.016
Glu	88.4^{a}	86.9^{ab}	87.7^{ab}	81.6^{d}	82.6^{cd}	84.9^{bc}	2.09	0.093	< 0.001	0.035
Gly	80.1	75.9	77.5	64.7	63.9	66.8	4.07	0.175	< 0.001	0.251
Pro	77.4	75.0	75.6	67.3	67.5	71.5	3.49	0.215	< 0.001	0.069
Ser	77.5	74.0	77.1	64.1	65.5	68.4	5.15	0.268	< 0.001	0.329
Tyr	79.1^{a}	75.5^{a}	77.0^{a}	68.3^{c}	70.0^{bc}	75.0^{ab}	3.30	0.029	< 0.001	0.003

Note: each least squares mean represents 8 observations.
$^{a\text{-}d}$Means within a row with different superscripts differ ($P < 0.05$).

Table 5.5 Basal ileal endogenous losses of crude protein (CP) and amino acids (AA) for broiler chickens and pigs, mg/kg dry matter intake (DMI)

Item	Broiler chickens	Pigs	SED	*P*-value
CP, g/kg DMI	8.00	17.32	0.957	< 0.001
Indispensable AA				
Arg	206	497	35.7	< 0.001
His	105	238	16.2	< 0.001
Ile	239	378	24.5	< 0.001
Leu	374	703	46.3	< 0.001
Lys	275	483	35.2	< 0.001
Met	62	86	7.9	0.010
Phe	237	427	26.6	< 0.001
Thr	446	686	45.6	< 0.001
Trp	44	58	17.2	0.427
Val	394	621	48.3	< 0.001
Dispensable AA				
Ala	255	551	36.7	< 0.001
Asp	487	958	69.0	< 0.001
Cys	155	293	22.5	< 0.001
Glu	580	1,164	85.3	< 0.001
Gly	289	1,240	74.1	< 0.001
Pro	318	609	60.0	0.003
Ser	302	1,934	306.8	0.003
Tyr	180	280	17.1	< 0.001

Note: each mean represents 8 observations for broiler chickens and 7 observations for pigs.

Table 5.6 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in full-fat canola seeds (FFCS), canola meal (CM), and canola expellers (CE) for broiler chickens and pigs

	Broiler chickens			Pigs				*P*-value		
Item	FFCS	CM	CE	FFCS	CM	CE	SD	Diet	Species	Diet × species
CP	80.2	77.7	79.4	74.9	76.0	80.3	3.46	0.055	0.050	0.054
Indispensable AA										
Arg	89.2^{a}	87.5^{abc}	88.4^{ab}	83.2^{c}	84.7^{bc}	88.0^{ab}	2.85	0.074	< 0.001	0.032
His	87.7^{a}	85.2^{ab}	86.5^{a}	81.7^{b}	83.8^{ab}	86.9^{a}	2.66	0.048	0.006	0.006
Ile	81.5^{a}	79.0^{ab}	79.8^{ab}	73.5^{c}	75.9^{bc}	80.3^{ab}	3.40	0.065	0.001	0.006
Leu	84.8^{a}	82.4^{ab}	83.2^{ab}	76.5^{c}	78.4^{bc}	83.1^{ab}	3.37	0.055	< 0.001	0.007
Lys	84.6	82.2	83.3	75.0	76.8	79.1	3.24	0.304	< 0.001	0.065
Met	88.0^{a}	86.9^{a}	87.8^{a}	80.7^{b}	84.5^{a}	87.2^{a}	2.32	0.003	< 0.001	< 0.001
Phe	84.2^{a}	82.9^{ab}	83.6^{ab}	75.7^{c}	78.9^{bc}	83.1^{ab}	3.38	0.023	< 0.001	0.008
Thr	78.5^{a}	75.3^{ab}	76.7^{ab}	72.1^{b}	73.9^{ab}	78.8^{a}	4.14	0.093	0.119	0.026
Trp	96.4^{a}	87.4^{b}	89.9^{b}	89.9^{b}	86.4^{b}	89.3^{b}	2.94	< 0.001	0.004	0.015
Val	79.3^{a}	76.8^{abc}	77.4^{ab}	71.1^{c}	72.9^{bc}	78.0^{ab}	3.93	0.099	0.002	0.013
Dispensable AA										
Ala	84.6^{a}	82.4^{ab}	83.0^{ab}	76.2^{c}	78.8^{bc}	82.1^{ab}	2.91	0.093	< 0.001	0.005
Asp	82.8^{a}	80.1^{abc}	81.4^{ab}	74.5^{c}	75.8^{bc}	80.2^{abc}	3.74	0.100	< 0.001	0.043
Cys	83.6^{a}	78.3^{b}	79.8^{ab}	77.7^{b}	77.6^{b}	80.9^{ab}	3.38	0.062	0.067	0.017
Glu	90.2^{a}	88.7^{ab}	89.5^{a}	85.1^{c}	86.3^{bc}	88.6^{ab}	2.09	0.081	< 0.001	0.031
Gly	83.3	79.1	80.7	78.4	78.0	80.7	4.07	0.218	0.095	0.222
Pro	81.9	79.6	80.2	75.9	76.3	80.4	3.49	0.182	0.005	0.062
Ser	80.7	77.1	80.1	84.5	85.1	87.2	5.15	0.392	< 0.001	0.483
Tyr	82.9^{a}	79.6^{ab}	80.9^{ab}	74.2^{c}	76.4^{bc}	81.0^{ab}	3.30	0.038	< 0.001	0.003

Note: each least squares mean represents 8 observations.

$^{a\text{-}c}$Means within a row with different superscripts differ ($P < 0.05$).

CHAPTER 6. COMPARATIVE AMINO ACID DIGESTIBILITY BETWEEN BROILER CHICKENS AND PIGS FED POULTRY BYPRODUCTS AND MEAT AND BONE MEAL

6.1 Abstract

The objective of this study was to compare the standardized ileal digestibility (SID) of amino acids (AA) in three poultry byproducts including hydrolyzed feather meal (HFM), flash dried poultry protein (FDPP), and poultry meal (PM) and meat and bone meal (MBM) between broiler chickens and pigs. Four experimental diets for both broiler chickens and pigs were prepared to contain each test ingredient as a sole source of nitrogen. A nitrogen-free diet was also prepared to determine the basal ileal endogenous losses (BEL) of AA. In Exp. 1, 416 male broiler chickens with mean initial body weight (BW) of 705 ± 100 g were allotted to 5 dietary treatments in a randomized complete block design with BW as a blocking factor at d 18 post hatching. After 5 d of feeding experimental diets, birds were euthanized by CO_2 asphyxiation, and ileal digesta samples were collected from distal two-thirds of the ileum. In Exp. 2, 10 barrows with a mean initial BW of 22.1 ± 1.59 kg were surgically fitted with T-cannulas at the distal ileum and allotted to a duplicate 5 × 4 incomplete Latin Square design with 5 dietary treatments and 4 periods. Each period lasted for 7 d including 5 d of adaptation and 2 d of ileal digesta collection. Data from Exp. 1 and 2 were pooled together and analyzed as a 2 × 4 factorial arrangement with the effects of species (i.e., broiler chickens or pigs) and four experimental diets (i.e., HFM, FDPP, PM, or MBM). There were interactions ($P < 0.05$) between experimental diets and species in the SID of His, Thr, Trp, Val, and dispensable AA, except for Tyr. In broiler chickens, the SID of His, Thr, and Trp in FDPP and PM were greater ($P < 0.05$) than in HFM but were less ($P < 0.05$) than MBM. In pigs however, there was no difference in SID of His, Thr, and Trp among FDPP, PM, and MBM, all of which were greater ($P < 0.05$) than the values in HFM. Broiler chickens fed the diet containing MBM had greater ($P < 0.05$) SID of Val than those fed the diets containing the other test ingredients, but there was no difference in the SID of Val among test ingredients in pigs. Pigs had greater ($P < 0.05$) SID of Arg, Ile, Leu, Met, and Phe than broiler chickens. The SID of Arg and Lys in FDPP and PM were greater ($P < 0.05$) than in HFM but were less ($P < 0.05$) than in MBM in both broiler chickens and pigs. In conclusion, the pattern of differences in the SID of AA among poultry byproducts and MBM were different between broiler chickens and pigs.

Key words: amino acid, digestibility, meat and bone meal, poultry, poultry byproducts, swine

6.2 Introduction

Broiler chickens and pigs are the primary non-ruminant animals raised for human consumption, and their diets typically contain common feed ingredients such as corn or soybean meal. In non-ruminant animals, most of digestion and absorption of protein and amino acids (**AA**) occur before digesta flows into the hindgut (Pond et al., 2005). Therefore, the standardized ileal digestibility (**SID**) of AA has been used to evaluate the digestibility of AA in feed ingredients for both broiler chickens and pigs (Kong and Adeola, 2014). However, differences in physical structure and capacity of digestive organs between broiler chickens and pigs may affect the SID of AA in the same feed ingredients. Park et al. (2017) reported that pigs had greater SID of AA in full-fat soybean, soybean meal, and peanut flour than broiler chickens, and the SID of most AA in soybean meal and peanut flour were greater than in full-fat soybean in both broiler chickens and pigs. On the other hand, another study conducted to compare the SID of AA between broiler chickens and pigs revealed that broiler chickens had greater SID of AA in full-fat canola seeds than pigs, which might be due to the beneficial effects of dietary fiber in full-fat canola seeds on the gizzard of broiler chickens (Park et al., 2019). Therefore, feed ingredient-specific factors may differently affect the SID of AA in broiler chickens and pigs.

Byproducts of meat production have been used to produce protein-rich feed ingredients for both broiler chickens and pigs. From poultry meat production, feathers separated from carcasses are generally autoclaved to hydrolyze keratin, which is insoluble and indigestible protein for non-ruminant animals, followed by processing to hydrolyzed feather meal (**HFM**; Papadopoulos, 1985). Inedible byproducts from poultry meat processing such as skin, bone, or trimmings are used to produce either flash dried poultry protein (**FDPP**) or poultry meal (**PM**), depending on the method of heat processing and proportion of bone. Meat and bone meal (**MBM**) is produced by mixture of byproducts from beef, pork, and poultry processing (Hicks and Verbeek, 2016). Nutrient composition as well as their digestibility in meat byproducts are affected by various composition, quality of raw materials, and processing conditions such as pressure and temperature (Hicks and Verbeek, 2016). However, it is unclear that the processing effects of meat byproducts on SID of AA are different between broiler chickens and pigs. Therefore, the objective of this study was to compare the SID of AA in HFM, FDPP, PM, and MBM between broiler chickens and pigs.

6.3 Materials and Methods

Experimental procedures involving animals were reviewed and approved by the Purdue University Animal Care and Use Committee.

6.3.1 Ingredients and Experimental Diets

Three poultry byproducts including HFM, FDPP, and PM and MBM (Darling Ingredient Inc., Cold Spring, KY) were obtained and used as test ingredients in the current study (Table 6.1). Five experimental diets were prepared based on cornstarch and sucrose (Table 6.2). Four experimental diets were formulated to contain each test ingredient as a sole source of nitrogen, providing 160 g/kg crude protein (**CP**) in each diet. A nitrogen-free diet (**NFD**) was prepared to estimate the basal ileal endogenous losses (**BEL**) of CP and AA in both broiler chickens and pigs. Soybean oil and purified cellulose were added into diets as energy and fiber sources, respectively. All diets were prepared to meet or exceed the vitamin and mineral requirement estimates for both broiler chickens and pigs suggested in National Research Council (**NRC**; 1994, 2012). Chromic oxide was added at 5 g/kg in experimental diets as an indigestible marker.

6.3.2 Experiment 1: Digestibility of Crude Protein and Amino Acids for Broiler Chickens

Male broiler chickens (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR) with mean body weight (**BW**) of 43.4 g were obtained from a commercial hatchery at d 0 post hatching. Birds were individually tagged with identification numbers and housed in electrically heated battery brooders (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR). The temperature of battery brooders was set at 35°C on d 0 post hatching and gradually decreased to 27°C during 22 d of housing. Birds were fed a corn-soybean meal-based standard starter diet containing 210 g CP/kg for 18 d. On d 18 post hatching, birds were individually weighed (mean initial BW = 705 ± 100 g), and 416 birds were assigned in a randomized complete block design with BW as a blocking factor to 5 dietary treatments. Each dietary treatment consisted of 8 replicates with 10 birds per cage for the diets containing test ingredients and with 12 birds per cage for NFD. Birds had free access to feed and water for 5 d. On d 23 post hatching, birds were euthanized by CO_2 asphyxiation, and ileum was excised for the collection of ileal digesta from distal two-thirds of the ileum (i.e., a portion of the small intestine from the Meckel's diverticulum

to ileocecal junction). Ileal digesta samples were pooled within cages and immediately stored at –20°C.

6.3.3 Experiment 2: Digestibility of Crude Protein and Amino Acids for Pigs

Ten barrows were surgically fitted with T-cannulas at the distal ileum as described by Dilger et al. (2004). After 7 d of recovery period, pigs (mean initial BW = 22.1 ± 1.59 kg) were divided into 2 blocks based on BW and assigned to a duplicate 5 × 4 incomplete Latin Square design with 5 dietary treatments and 4 periods. Pigs were individually housed in metabolism crates (1.22 × 1.22 m) equipped with a feeder and a nipple drinker during the experimental periods.

Individual BW of pigs was measured at the beginning of each period to calculate the daily feed allowance at 4% of mean BW within blocks, which was divided into 2 equal meals and provided to pigs at 0800 and 1700 h. Each experimental period consisted of 5 d of adaptation and 2 d of ileal digesta collection. During collection, ileal digesta samples were collected with plastic sample bags (Whirl-Pak bag; NASCO, Fort Atkinson, WI) attached to T-cannulas from 0830 to 1700 h. Plastic samples bags were prepared to contain 10 mL of 10% formic acid and changed every 30 min. Collected ileal digesta samples were immediately stored at –20°C. At the end of experiment, frozen ileal digesta samples were slightly thawed and pooled within pigs and diets. Approximately 300 mL was subsampled from pooled and homogenized ileal digesta samples and stored at –20°C.

6.3.4 Chemical Analysis

Ileal digesta samples collected from broiler chickens and pigs were freeze-dried before chemical analyses. Test ingredients, experimental diets, and ileal digesta samples were finely ground through 0.75 mm screen using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany). Ground samples were analyzed for dry matter by drying at 105°C for 24 h in a forced-air drying oven [Precision Scientific Co., Chicago, IL; method 934.01; Association of Official Analytical Chemists (**AOAC**), 2006]. The concentration of nitrogen in ground samples was analyzed by a combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the concentration CP was calculated as the product of nitrogen concentration and 6.25. Ground samples were analyzed for AA at University of Missouri Agricultural

Experiment Station Chemical Laboratories (Columbia, MO). For the preparation of AA analysis, ground samples were hydrolyzed by 6 M HCl (or BaOH for Trp analysis) at 110°C for 24 h under nitrogen atmosphere. For the analysis of Met and Cys, samples were oxidized by performic acid before acid hydrolysis. The concentration of AA in samples were analyzed by a high-performance liquid chromatography after post-column derivatization [method 982.30 E (a, b, c); AOAC, 2006]. Ground samples of test ingredients were analyzed for acid hydrolyzed ether extract (method 954.02; AOAC, 2000) and ash (method 942.05; AOAC, 2006). The concentration of Cr in ground experimental diets and ileal digesta samples were measured by a spectrophotometry (Spark 10M; Tecan Group Ltd., Männedorf, Switzerland) at 450 nm of absorption after the wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

6.3.5 Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and SID (%) of CP and AA in test ingredients and BEL of CP and AA [mg/kg dry matter intake (**DMI**)] in broiler chickens and pigs were calculated as described in Park et al. (2017).

Data outside of 2.5 times interquartile range were considered outliers before statistical analysis. Data from Exp. 1 and 2 were pooled and considered a 2 × 4 factorial arrangement with the effects of species (broiler chickens or pigs) and four experimental diets (HFM, FDPP, PM, or MBM). Data were analyzed by ANOVA using general linear models (SAS Inst. Inc., Cary, NC). The model included species, experimental diet, interaction between species and experimental diet, and block within species as independent variables. The components of Latin Square design in Exp. 2 were rearranged as described in Park et al. (2019) to match the blocking factors in the model. If interactions were observed, least squares means of experimental diets and species were separated by the pairwise comparison with the Tukey's adjustment. In the absence of interaction, least squares means of significant main effects of experimental diets were separated by the pairwise comparison with the Tukey's adjustment. Experimental unit was cage for Exp. 1 and pig for Exp. 2. Significance of the model and differences among means were set at $P < 0.05$.

6.4 Results

Animals used in all experiments were in good condition. Data from two pigs in Exp. 2 (one assigned to the diet containing HFM and the other one assigned to FDPP) were outliers as they were outside 2.5 times interquartile range and thus excluded from dataset in statistical analysis.

6.4.1 Chemical Analysis

On an as-fed basis, the concentration of CP in test ingredients ranged from 500 g/kg for PM to 890 g/kg for HFM (Table 6.1). The concentration of ash in PM was 290 g/kg on an as-fed basis, whereas that in FDPP was 125 g/kg on an as-fed basis. Hydrolyzed feather meal contained the least concentration of ash at 36.3 g/kg on an as-fed basis. The concentration of CP and AA in experimental diets were close to the values calculated using analyzed concentration of CP and AA in test ingredients (Table 6.3).

6.4.2 Apparent Ileal Digestibility of Crude Protein and Amino Acids

Interactions ($P < 0.05$) between experimental diets and species were observed in the AID of CP and AA, except for Arg, Ile, Met, Pro, and Tyr (Table 6.4). In broiler chickens, the AID of CP, Leu, Lys, and Phe in MBM were greater ($P < 0.05$) than in HFM and FDPP but were not different from the values in PM. The AID of His, Thr, Trp, and Val in MBM were greater ($P < 0.05$) than in the other feed ingredients within broiler chickens. In pigs however, the AID of CP, Lys, and Thr in FDPP were greater ($P < 0.05$) than HFM but were not different from the values in PM and MBM. Pigs fed the diet containing HFM had less ($P < 0.05$) AID of His and Trp than those fed the diets containing FDPP, PM, or MBM. The AID of Leu in MBM was greater ($P < 0.05$) than in HFM for pigs. No difference in AID of Phe and Val was observed among test ingredients within pigs.

6.4.3 Standardized Ileal Digestibility of Crude Protein and Amino Acids

The BEL of CP and AA in pigs were greater ($P < 0.05$) than in broiler chickens (Table 6.5). The BEL of indispensable AA ranged from 44 mg/kg DMI for Trp to 432 mg/kg DMI for Val in broiler chickens and from 105 mg/kg DMI for Met and 916 mg/kg DMI for Arg in pigs.

There were interactions ($P < 0.05$) between experimental diets and species in the SID of CP, His, Thr, Trp, Val, and dispensable AA, except for Tyr (Table 6.6). Broiler chickens fed the diets containing HFM and FDPP had less ($P < 0.05$) SID of CP than those fed the diets containing MBM, which was not different from the value in PM, whereas pigs fed the diets containing HFM had less ($P < 0.05$) SID of CP than those fed the diets containing the other test ingredients. In broiler chickens, the SID of His, Thr, and Trp in FDPP and PM were greater ($P < 0.05$) than in HFM but were less ($P < 0.05$) than MBM. In pigs however, there was no difference in SID of His, Thr, and Trp among FDPP, PM, and MBM, all of which were greater ($P < 0.05$) than the values in HFM. Broiler chickens fed the diet containing MBM had greater ($P < 0.05$) SID of Val than those fed the diets containing the other test ingredients, but there was no difference in the SID of Val among test ingredients in pigs. Pigs had greater ($P < 0.05$) SID of Arg, Ile, Leu, Met, and Phe than broiler chickens, but the SID of Lys for pigs was less ($P < 0.001$) than the value for broiler chickens. In both broiler chickens and pigs, the SID of Arg and Lys in FDPP and PM were greater ($P < 0.05$) than in HFM but were less ($P < 0.05$) than in MBM. The SID of Ile, Leu, Met, and Phe in HFM and FDPP were less ($P < 0.05$) than the values in MBM for both broiler chickens and pigs.

6.5 Discussion

Hydrolyzed feather meal mostly consists of feathers from poultry processing, whereas FDPP, PM, and MBM are produced from various byproducts including bone (Hicks and Verbeek, 2016). Therefore, HFM contained greater CP but less ash concentration compared with the other test ingredients. In addition, because keratin is rich in Cys which mainly forms disulfide bonds (Papadopoulos, 1985), the concentration of Cys in HFM was greater than the other test ingredients. The concentration of CP and AA in HFM were within the range of previously reported values (Bandegan et al., 2010; NRC, 2012; Sulabo et al., 2013). The concentration of CP and most AA in FDPP were greater but the concentration of ash in FDPP was less than in PM, probably due to reduced bone contents in FDPP. Published information on the nutrient composition as well as digestibility of nutrients in FDPP is limited, but the concentrations of CP and AA in FDPP are comparable with the values in poultry protein meal (Davies et al., 2019). However, the concentrations of CP and AA in PM are less than the values reported in previous studies (NRC, 2012; Rojas and Stein, 2013; Yoo et al., 2019). The greater concentration of ash in PM used in the current study compared with previous reports (NRC, 2012; Rojas and Stein, 2013; Yoo et al., 2019)

is perhaps due to bone contents and may account for the differences. The concentrations of CP and AA in MBM are within the range of previously reported values (NRC, 2012; Adeola et al., 2018; Navarro et al., 2018).

Park et al. (2019) reported greater BEL of CP and AA, except for Trp, in pigs than broiler chickens and discussed that it may be due to the differences in the length of small intestine between broiler chickens and pigs. The results of the current study are consistent with the findings of Park et al. (2019) that pigs had greater BEL of CP and AA compared with broiler chickens. The BEL of CP and AA in pigs agree with the values reported in previous studies (Navarro et al., 2018; Wang et al., 2018; Park et al., 2019). The BEL of CP and AA in broiler chickens are close to the values reported by Park et al. (2019) but less than the values reported in other studies (Kong and Adeola, 2013a,b; Park et al., 2017; Osho et al., 2019), especially for the BEL of Arg, Leu, Lys, and Phe. Differences in ingredient composition of NFD or in strain of broiler chickens among studies may be responsible. Nitrogen-free diet used in the current study and Park et al. (2019) were prepared based on sucrose and cornstarch, whereas NFD in the other studies were prepared based on dextrose and cornstarch (Kong and Adeola, 2013a,b; Park et al., 2017; Osho et al., 2019). Kong and Adeola (2013c) reported that broiler chickens fed NFD containing dextrose without cornstarch had greater BEL of CP and most AA than those fed NFD containing dextrose and cornstarch or cornstarch without dextrose. The source of carbohydrates in NFD may affect the BEL of CP and AA in broiler chickens. In addition, Cobb 500 broiler chickens were used in both Park et al. (2019) and the current study; however, Ross 708 (Kong and Adeola, 2013a,b; Park et al., 2017) or Ross 308 (Osho et al., 2019) broiler chickens were used in other studies. Kim and Corzo (2012) reported that the AID of most AA in animal byproduct blend were different between two strains of broiler chickens. Because the AID of AA was not corrected for the BEL of AA, it may be speculated that the BEL of AA is affected by strains of broiler chickens, leading to a different AID of AA in the same feed ingredient. Further research is needed to verify the differences in BEL of AA as well as digestibility of AA among strains of broiler chickens.

The SID of Arg, Ile, Leu, Met, and Phe in test ingredients for pigs were greater than broiler chickens, which is consistent with Park et al. (2017) which reported that the SID of most AA in full-fat soybean, soybean meal, and peanut flour for pigs were greater than the values for broiler chickens. On the other hand, interactions between experimental diets and species were observed in the SID of CP, His, Thr, Trp, Val, and dispensable AA, except for Tyr. This finding was similar

with the results observed by Park et al. (2019) in which there were interactions in the SID of most AA between experimental diets containing canola products and species of non-ruminant animals. In the current study, interactions were mainly observed because the SID of several AA in FDPP and PM were less than in MBM for broiler chickens, but not for pigs. The reason for this difference remains unclear. Perhaps heat damage during processing of FDPP and PM reduced the SID of several AA for broiler chickens, whereas in pigs, the extent of heat damage was inadequate to reduce the SID of several AA in FDPP and PM relative to MBM. Shirley and Parsons (2000) reported that the true ileal digestibility of AA in MBM determined by precision-fed cecectomized roosters decreased with increasing pressure and temperature during processing of MBM. Bellagamba et al. (2015) also reported that the in vitro protein digestibility of processed poultry protein was reduced by heat processing compared with freeze-drying. However, information for the comparison of critical temperature of meat byproduct processing between broiler chickens and pigs is limited. Batterham and Darnell (1986) reported that the availability of Lys in MBM determined by the slope-ratio assay with feed efficiency as responses decreased as the cooking temperature increased in both broiler chickens and pigs, but the extent of the reduction in the availability of Lys was similar between broiler chickens and pigs. Park et al. (2017) discussed that pigs may have greater digestibility of AA than broiler chickens due to the slower passage rate of digesta and longer small intestine in pigs compared with broiler chickens. Therefore, the ability of pigs to digest and utilize AA in heat-damaged proteins may be greater than that of broiler chickens. Further research is needed to verify the critical temperature during the processing for digestibility of AA in FDPP and PM fed to broiler chickens and pigs.

In both broiler chickens and pigs, the SID of most AA in HFM were less than in the other test ingredients. Papadopoulos (1985) discussed that prolonged autoclaving to hydrolyze keratins may denature proteins resistant to endogenous proteolytic enzymes. On the other hand, incomplete hydrolysis of keratin from inadequate heat processing may also reduce the digestibility of AA in HFM. Therefore, reduced SID of most AA in HFM compared with the other test ingredients could be due to the heat damage during hydrolysis or incomplete hydrolysis of keratin in HFM. Due to the difficulties in optimizing the temperature and processing time of HFM to maximize the hydrolysis with minimal heat damage, the digestibility of AA in HFM may vary among sources with different processing conditions (Wang and Parsons, 1997). Nevertheless, the SID of AA in

HFM determined in the current study agree with the previously reported values for broiler chickens (Bandegan et al., 2010) and for pigs (Sulabo et al., 2013).

No difference was observed in the SID of CP and most AA between FDPP and PM for both broiler chickens and pigs, even though PM contained greater concentration of ash than FDPP. Johnson et al. (1998) reported that the concentration of ash in poultry byproduct meal did not affect the true ileal digestibility of AA determined by precision-fed cecectomized rooster assay. Therefore, it may be concluded that protein and AA contents derived from poultry meal byproducts have similar digestibility values regardless of the inclusion rate of bone contents. Similar with the results of the current study, Rojas and Stein (2013) found that the SID of CP and most AA in chicken meal was not different from the values in poultry byproduct meal (**PBM**) when fed to pigs. However, it should be noted that the standardized ileal digestible AA contents in PM are less than the values in FDPP due to the dilution of proteins by increased bone contents.

Hicks and Verbeek (2016) reported that the difference between PM and PBM is the necks, feet, undeveloped eggs, and viscera, which are included in PBM, but not in PM. However, due to the large variation in byproduct composition and processing among sources of PM or PBM, difference in nutrient composition between PM and PBM is not apparent (Hicks and Verbeek, 2016). The SID of AA in PM for broiler chickens determined in the current study are within the range of SID of AA in 5 different sources of PBM (Bandegan et al., 2010). However, the SID of most AA in PM for broiler chickens are greater than the values in PBM reported in Kim et al. (2011). Moreover, the SID of most AA in PM for pigs determined in the current study were greater than the values in chicken meal and PBM reported in Rojas and Stein (2013) but were less the values in poultry byproduct suggested in NRC (2012). This discrepancy may be due to the differences in temperature and pressure during processing of products or in composition of byproducts. Hicks and Verbeek (2016) suggested that the quality of meat byproducts is dependent on various factors including the proportion, types, and characteristics of starting raw material and processing conditions including pressure of steam, temperature during agitation and drying.

Unlike the other test ingredients which exclusively contain byproducts from poultry meat production, MBM is produced by rendering of byproducts originating from general meat production including beef, pork, and poultry (Hick and Verbeek, 2016). Therefore, MBM may be more susceptible to variation in digestibility of nutrients. Nevertheless, the SID of most AA in MBM are within the range of previously reported values for broiler chickens (Adedokun et al.,

2007; Rochell et al., 2013; Adedokun et al., 2014) and for pigs (NRC, 2012; Navarro et al., 2018; Wang et al., 2018). However, it should be noted that previous studies in broiler chickens or pigs investigating the nutritional quality of MBM outnumber the other test ingredients used in the current study that provide a wide range of SID values.

In conclusion, there were interactions in the SID of CP, His, Thr, Trp, Val, and dispensable AA, except for Tyr between meals from rendering and species, most of which were due to the lower SID values in flash dried poultry protein and poultry meal than meat and bone meal for broiler chickens. Pigs had greater SID of Arg, Ile, Leu, Met, and Phe in hydrolyzed feather meal, flash dried poultry protein, poultry meal, and meat and bone meal than broiler chickens. In both broiler chickens and pigs, the SID of most AA in hydrolyzed feather meal were less than in the other test ingredients, and those in flash dried poultry protein were not different from the values in poultry meal.

6.6 Literature Cited

Adedokun, S. A., P. Jaynes, M. E. Abd El-Hack, R. L. Payne, and T. J. Applegate. 2014. Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. Poult. Sci. 93:420-428. doi:10.3382/ps.2013-03495

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chickens and turkey poults with a nitrogen-free or casein diet. Poult. Sci. 86:2598-2607. doi:10.3382/ps.2007-00164

Adeola, O., M. N. Anwar, M. R. Abdollahi, and V. Ravindran. 2018. Age-related energy values of meat and bone meal for broiler chickens. Poult. Sci. 97:2516-2524. doi:10.3382/ps/pey100

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bandegan, A., E. Kiarie, R. L. Payne, G. H. Crow, W. Guenter, and C. M. Nyachoti. 2010. Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. Poult. Sci. 89:2626-2633. doi:10.3382/ps.2010-00757

Batterham, E. S., and R. E. Darnell. 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal as determined by slope-ratio assays with growing pigs, rats and chicks and by chemical techniques. Br. J. Nutr. 55:441-453. doi:10.1079/bjn19860050

Bellagamba, F., F. Caprino, T. Mentasti, M. Vasconi, and V. M. Moretti. 2015. The impact of processing on amino acid racemization and protein quality in processed animal proteins of poultry origin. Ital. J. Anim. Sci. 14:3770. doi:10.4081/ijas.2015.3770

Davies, S. J., J. Laporte, A. Gouveia, H. S. Salim, S. L. Woodgate, M. S. Hassaan, and E. R. El-Haroun. 2019. Validation of processed animal proteins (mono-PAPS) in experimental diets for juvenile gilthead sea bream (*Sparus aurata* L.) as primary fish meal replacers within a European perspective. Aquacult. Nutr. 25:225-238. doi:10.1111/anu.12846

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724. doi:10.2527/2004.823715x

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634. doi:10.4141/cjas79-081

Hicks, T. M., and C. J. R. Verbeek. 2016. Meat industry protein by-products: Sources and characteristics. In: G. S. Dhillon, editor, Protein byproducts: Transformation from environmental burden into value-added products. Academic Press, London, United Kingdom. p. 37-61. doi:10.1016/B978-0-12-802391-4.00003-3

Johnson, M. L., C. M. Parsons, G. C. Fahey Jr., N. R. Merchen, and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J. Anim. Sci. 76:1112-1122. doi:10.2527/1998.7641112x

Kim, E. J., and A. Corzo. 2012. Interactive effects of age, sex, and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. Poult. Sci. 91:908-917. doi:10.3382/ps.2011-01820

Kim, E. J., P. L. Utterback, T. J. Applegate, and C. M. Parsons. 2011. Comparison of amino acid digestibility of feedstuffs determined with the precision-fed cecectomized rooster assay and the standardized ileal amino acid digestibility assay. Poult. Sci. 90:2511-2519. doi:10.3382/ps.2011-01400

Kong, C., and O. Adeola. 2013a. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Pekin ducks. Poult. Sci. 92:2381-2388. doi:10.3382/ps.2013-03179

Kong, C., and O. Adeola. 2013b. Comparative amino acid digestibility for broiler chickens and White Pekin ducks. Poult. Sci. 92:2367-2374. doi:10.3382/ps.2013-03042

Kong, C., and O. Adeola. 2013c. Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in broiler chickens. Poult. Sci. 92:1276-1282. doi:10.3382/ps.2012-02835

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Navarro, D. M. D. L., J. K. Mathai, N. W. Jaworski, and H. H. Stein. 2018. Amino acid digestibility in six sources of meat and bone meal, blood meal, and soybean meal fed to growing pigs. Can. J. Anim. Sci. 98:860-867. doi:10.1139/cjas-2017-021

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333-1340. doi:10.3382/ps/pey457

Papadopoulos, M. C. 1985. Processed chicken feathers as feedstuff for poultry and swine. A review. Agric. Wastes 14:275-290. doi:10.1016/S0141-4607(85)80009-3

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110-3119. doi:10.2527/jas2017.1404

Park, C. S., D. Ragland, A. Helmbrecht, J. K. Htoo, and O. Adeola. 2019. Digestibility of amino acid in full-fat canola seeds, canola meal, and canola expellers fed to broiler chickens and pigs. J. Anim. Sci. 97:803-812. doi:10.1093/jas/sky470

Pond, W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2005. Basic animal nutrition and feeding. 5th ed. John Wiley & Sons, Inc., Hoboken, NJ.

Rochell, S. J., D. L. Kuhlers, and W. A. Dozier III. 2013. Relationship between in vitro assays and standardized ileal amino acid digestibility of animal protein meals in broilers. Poult. Sci. 92:158-170. doi:10.3382/ps.2012-02365

Rojas, O. J., and H. H. Stein. 2013. Concentration of digestible and metabolizable energy and digestibility of amino acids in chicken meal, poultry byproduct meal, hydrolyzed porcine intestines, a spent hen-soybean meal mixture, and conventional soybean meal fed to weanling pigs. J. Anim. Sci. 91:3220-3230. doi:10.2527/jas2013-6269

Shirley, R. B., and C. M. Parsons. 2000. Effect of pressure processing on amino acid digestibility of meat and bone meal for poultry. Poult. Sci. 79:1775-1781. doi:10.1093/ps/79.12.1775

Sulabo, R. C., L. I. Chiba, F. N. Almeida, S. D. Brotzge, R. L. Payne, and H. H. Stein. 2013. Amino acid and phosphorus digestibility and concentration of digestible and metabolizable energy in hydrolyzed feather meal fed to growing pigs. J. Anim. Sci. 91:5829-5837. doi:10.2527/jas2013-6267

Wang, T., S. O. Osho, and O. Adeola. 2018. 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. J. Anim. Sci. 96:4731-4742. doi:10.1093/jas/sky326

Wang, X., and C. M. Parsons. 1997. Effect of processing systems on protein quality of feather meals and hog hair meals. Poult. Sci. 76:491-496. doi:10.1093/ps/76.3.491

Yoo, J. S., K. H. Cho, J. S. Hong, H. S. Jang, Y. H. Chung, G. T. Kwon, D. G. Shin, and Y. Y. Kim. 2019. Nutrient ileal digestibility evaluation of dried mealworm (*Tenebrio molitor*) larvae compared to three animal protein by-products in growing pigs. Asian-Australas. J. Anim. Sci. 32:387-394. doi:10.5713/ajas.18.0647

Table 6.1 Analyzed nutrient composition of hydrolyzed feather meal (HFM), flash dried poultry protein (FDPP), poultry meal (PM), and meat and bone meal (MBM), g/kg as-fed basis

	Ingredient			
Item	HFM	FDPP	PM	MBM
Dry matter	958	954	933	965
Gross energy, kcal/kg	5,436	5,105	4,080	4,029
Crude protein[1]	890	695	500	548
AEE[2]	90.8	146.1	158.8	105.3
Ash	36.3	125	290	280
Indispensable amino acid				
Arg	62.9	44.4	33.6	34.9
His	7.2	13.7	7.5	11.0
Ile	41.8	25.9	14.5	14.8
Leu	71.2	45.7	26.2	32.8
Lys	20.1	40.9	24.6	29.0
Met	7.0	13.0	7.9	6.8
Phe	42.8	26.7	16.8	18.9
Thr	41.1	25.5	15.3	16.7
Trp	5.2	6.4	3.0	4.3
Val	69.5	32.3	18.6	23.8
Dispensable amino acid				
Ala	41.7	42.3	35.7	38.7
Asp	59.1	52.9	34.8	39.6
Cys	48.1	8.2	3.4	4.9
Glu	92.1	80.8	56.6	61.6
Gly	70.1	60.3	64.8	67.7
Pro	88.0	40.7	38.4	41.1
Ser	86.3	24.5	14.8	19.0
Tyr	23.6	20.9	10.8	11.4

[1]Analyzed nitrogen concentration multiplied by 6.25.
[2]AEE = acid hydrolyzed ether extract.

Table 6.2 Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[1]				
Item	HFM	FDPP	PM	MBM	NFD
Cornstarch	351.2	332.2	243.0	286.0	316.0
Sucrose	300.0	300.0	300.0	300.0	500.0
Hydrolyzed feather meal	181.0	0.0	0.0	0.0	0.0
Flash dried poultry protein	0.0	226.0	0.0	0.0	0.0
Poultry meal	0.0	0.0	323.0	0.0	0.0
Meat and bone meal	0.0	0.0	0.0	280.0	0.0
Soybean oil	50.0	50.0	50.0	50.0	50.0
Cellulose[2]	50.0	50.0	50.0	50.0	50.0
Ground limestone	12.3	0.8	0.0	0.0	13.0
Monocalcium phosphate	21.5	7.0	0.0	0.0	23.5
Salt	4.0	4.0	4.0	4.0	0.0
Potassium carbonate	0.0	0.0	0.0	0.0	2.6
Magnesium oxide	0.0	0.0	0.0	0.0	2.0
Sodium bicarbonate	0.0	0.0	0.0	0.0	7.5
Choline chloride	0.0	0.0	0.0	0.0	2.5
Potassium chloride	0.0	0.0	0.0	0.0	2.9
Vitamin-mineral premix[3]	5.0	5.0	5.0	5.0	5.0
Chromic oxide premix[4]	25.0	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000	1,000

[1]HFM = hydrolyzed feather meal; FDPP = flash dried poultry protein; PM = poultry meal; MBM = meat and bone meal; NFD = nitrogen-free diet.

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

[3]Provided the following quantities per kilogram 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; and Se, 0.43 mg.

[4]Provided 5 g chromic oxide/kg of complete diet.

Table 6.3 Analyzed concentration of dry matter, crude protein (CP), and amino acids (AA) in experimental diets, g/kg as-fed basis

	Diet[1]				
Item	HFM	FDPP	PM	MBM	NFD
Dry matter	947	950	954	954	963
CP[2]	168	157	159	149	10.9
Indispensable AA					
Arg	10.4	9.4	10.2	9.6	0.0
His	1.2	2.9	2.3	3.0	0.0
Ile	7.8	5.8	4.5	4.1	0.0
Leu	12.7	10.2	8.3	9.3	0.1
Lys	3.7	8.9	7.6	8.0	0.1
Met	1.2	2.8	2.4	1.9	0.1
Phe	7.6	5.8	5.2	5.4	0.1
Thr	7.1	5.6	4.8	4.7	0.0
Trp	0.9	1.4	1.0	1.2	0.0
Val	12.3	7.3	5.9	6.8	0.1
Disposable AA					
Ala	7.4	9.7	11.4	11.2	0.1
Asp	10.4	11.8	11.0	11.2	0.1
Cys	8.2	1.9	1.1	1.3	0.0
Glu	16.9	18.7	18.1	17.6	0.1
Gly	12.5	13.7	20.6	19.5	0.1
Pro	15.6	9.0	12.3	12.0	0.2
Ser	14.7	5.5	4.6	5.1	0.1
Tyr	3.6	3.8	3.0	2.9	0.0

[1]HFM = hydrolyzed feather meal; FDPP = flash dried poultry protein; PM = poultry meal; MBM = meat and bone meal; NFD = nitrogen-free diet.
[2]Analyzed nitrogen multiplied by 6.25.

Table 6.4 Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in hydrolyzed feather meal (HFM), flash dried poultry protein (FDPP), poultry meal (PM), and meat and bone meal (MBM) fed to broiler chickens and pigs

	Broiler chickens				Pigs					*P*-value		
Item	HFM	FDPP	PM	MBM	HFM	FDPP	PM	MBM	SD	Diet	Species	Diet × species
CP	60.8^{cd}	67.4^{bc}	71.8^{ab}	77.3^{a}	49.7^{e}	59.4^{cd}	57.1^{de}	55.9^{de}	4.95	< 0.001	< 0.001	0.004
Indispensable AA												
Arg	67.2^{z}	75.3^{y}	82.0^{x}	86.7^{x}	62.6^{z}	73.8^{y}	75.4^{x}	77.8^{x}	3.95	< 0.001	< 0.001	0.089
His	49.3^{d}	66.7^{c}	72.2^{bc}	81.6^{a}	44.9^{d}	67.3^{bc}	68.0^{bc}	73.4^{b}	4.05	< 0.001	< 0.001	0.044
Ile	71.1^{x}	65.6^{y}	70.8^{xy}	74.5^{x}	73.5^{x}	68.6^{y}	69.9^{xy}	71.1^{x}	3.93	0.001	0.790	0.112
Leu	65.9^{c}	68.2^{c}	74.4^{ab}	80.0^{a}	67.5^{c}	69.6^{bc}	70.6^{bc}	74.5^{ab}	3.82	< 0.001	0.129	0.027
Lys	50.7^{e}	71.2^{bcd}	76.2^{ab}	84.2^{a}	25.6^{f}	64.5^{cd}	63.6^{d}	73.0^{bc}	5.35	< 0.001	< 0.001	< 0.001
Met	56.5^{z}	72.9^{y}	77.4^{xy}	78.2^{x}	58.7^{z}	75.7^{y}	76.4^{xy}	78.1^{x}	3.63	< 0.001	0.298	0.410
Phe	67.3^{c}	67.9^{c}	75.1^{ab}	79.4^{a}	69.1^{bc}	69.2^{bc}	71.7^{bc}	74.7^{ab}	3.71	< 0.001	0.209	0.046
Thr	52.8^{d}	61.4^{bc}	65.8^{b}	72.4^{a}	52.7^{d}	60.3^{bc}	58.1^{cd}	59.0^{cd}	4.00	< 0.001	< 0.001	< 0.001
Trp	64.9^{c}	79.8^{b}	80.1^{b}	89.7^{a}	50.5^{d}	76.9^{b}	75.9^{b}	77.2^{b}	5.05	< 0.001	< 0.001	0.005
Val	65.8^{b}	62.6^{b}	66.7^{b}	76.5^{a}	67.2^{b}	64.2^{b}	63.0^{b}	67.6^{b}	4.20	< 0.001	0.031	0.004
Dispensable AA												
Ala	64.3^{de}	72.8^{c}	80.4^{ab}	87.1^{a}	57.5^{e}	70.0^{cd}	71.4^{c}	75.6^{bc}	4.22	< 0.001	< 0.001	0.048
Asp	35.5^{e}	52.7^{bc}	58.5^{b}	74.5^{a}	30.4^{e}	49.3^{cd}	44.2^{d}	56.8^{bc}	4.70	< 0.001	< 0.001	< 0.001
Cys	44.0^{a}	43.4^{a}	33.0^{bc}	26.5^{cd}	41.1^{ab}	46.8^{a}	38.8^{ab}	21.1^{d}	5.91	< 0.001	0.868	0.039
Glu	57.1^{d}	69.8^{bc}	75.6^{b}	82.1^{a}	55.0^{d}	69.3^{bc}	68.2^{c}	71.1^{bc}	4.04	< 0.001	< 0.001	0.003
Gly	63.3^{b}	70.5^{b}	79.6^{a}	88.4^{a}	48.9^{c}	61.5^{b}	64.6^{b}	66.0^{b}	5.66	< 0.001	< 0.001	0.022
Pro	57.6^{z}	65.0^{yz}	75.9^{x}	84.9^{xy}	-14.6^{z}	2.8^{yz}	32.9^{x}	21.0^{xy}	19.69	< 0.001	< 0.001	0.224
Ser	62.3^{bc}	61.0^{bcd}	63.6^{b}	73.4^{a}	61.5^{bcd}	59.5^{bcd}	55.0^{d}	57.0^{cd}	4.04	0.001	< 0.001	< 0.001
Tyr	60.8^{y}	69.1^{x}	69.5^{x}	74.2^{x}	59.0^{y}	68.8^{x}	67.4^{x}	67.1^{x}	4.42	< 0.001	0.018	0.187

Note: each least squares mean represents 8 observations except for pigs fed HFM and FDPP (7 observations).
[a-f]Means within a row with different superscripts differ ($P < 0.05$).
[x-z]Means within a row with different superscripts within species differ ($P < 0.05$).

Table 6.5 Basal ileal endogenous losses of crude protein (CP) and amino acids (AA) for broiler chickens and pigs

Item	Broiler chickens	Pigs	SED	*P*-value
CP, g/kg DMI[1]	8.52	27.88	2.29	< 0.001
Indispensable AA, mg/kg DMI				
Arg	172	916	95.3	< 0.001
His	80	234	24.0	< 0.001
Ile	204	394	49.5	0.004
Leu	273	704	82.5	< 0.001
Lys	189	629	81.2	< 0.001
Met	60	105	14.4	0.011
Phe	181	420	50.8	0.001
Thr	396	766	85.5	0.003
Trp	44	132	14.1	< 0.001
Val	432	751	73.4	< 0.001
Dispensable AA, mg/kg DMI				
Ala	211	768	67.9	< 0.001
Asp	416	1081	124.2	< 0.001
Cys	158	250	31.8	0.020
Glu	487	1,356	177.3	0.001
Gly	251	2,008	200.2	< 0.001
Pro	295	8,118	994.8	< 0.001
Ser	303	715	61.3	< 0.001
Tyr	133	300	38.2	0.002

Note: each mean represents 8 observations.
[1]DMI = dry matter intake.

Table 6.6 Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in hydrolyzed feather meal (HFM), flash dried poultry protein (FDPP), poultry meal (PM), and meat and bone meal (MBM) fed to broiler chickens and pigs

	Broiler chickens				Pigs					*P*-value		
Item	HFM	FDPP	PM	MBM	HFM	FDPP	PM	MBM	SD	Diet	Species	Diet × species
CP	66.1^{c}	73.0^{bc}	77.4^{ab}	83.2^{a}	66.9^{c}	77.9^{ab}	75.3^{ab}	75.3^{b}	4.95	< 0.001	0.412	0.008
Indispensable AA												
Arg	68.9^{z}	77.2^{y}	83.7^{y}	88.5^{x}	71.7^{z}	83.9^{y}	84.7^{y}	87.7^{x}	3.95	< 0.001	0.024	0.075
His	56.2^{e}	69.6^{cd}	75.8^{bc}	84.4^{a}	65.1^{d}	75.7^{bc}	78.5^{ab}	81.5^{ab}	4.05	< 0.001	0.001	0.002
Ile	73.8^{yz}	69.3^{z}	75.5^{xy}	79.7^{x}	78.7^{yz}	75.6^{z}	79.0^{xy}	81.1^{x}	3.93	< 0.001	< 0.001	0.374
Leu	68.1^{z}	70.9^{z}	77.8^{y}	83.0^{x}	73.3^{z}	76.8^{z}	79.4^{y}	82.4^{x}	3.82	< 0.001	0.004	0.080
Lys	56.0^{z}	73.4^{y}	78.8^{y}	86.7^{x}	43.3^{z}	71.8^{y}	72.2^{y}	81.1^{x}	5.35	< 0.001	< 0.001	0.053
Met	61.7^{z}	75.1^{y}	80.0^{xy}	81.5^{x}	67.8^{z}	79.6^{y}	80.9^{xy}	83.8^{x}	3.63	< 0.001	< 0.001	0.223
Phe	69.7^{y}	71.1^{y}	78.7^{x}	82.9^{x}	74.9^{y}	76.7^{y}	80.1^{x}	82.8^{x}	3.71	< 0.001	0.003	0.118
Thr	58.6^{e}	68.7^{cd}	74.4^{bc}	81.1^{a}	64.0^{de}	74.5^{abc}	74.7^{bc}	75.9^{ab}	4.00	< 0.001	0.139	0.001
Trp	69.8^{c}	83.0^{b}	84.6^{b}	93.5^{a}	65.3^{c}	86.5^{ab}	89.8^{ab}	88.7^{ab}	5.05	< 0.001	0.900	0.012
Val	69.5^{cd}	68.8^{d}	74.3^{bcd}	83.1^{a}	73.5^{bcd}	74.8^{bcd}	76.2^{bc}	79.1^{ab}	4.20	< 0.001	0.074	0.012
Dispensable AA												
Ala	67.3^{e}	75.1^{cd}	82.3^{b}	89.1^{a}	68.3^{de}	78.2^{bc}	78.4^{bc}	82.7^{ab}	4.22	< 0.001	0.159	0.014
Asp	39.7^{e}	56.4^{cd}	62.4^{bc}	78.4^{a}	41.1^{e}	58.8^{cd}	54.4^{d}	66.9^{b}	4.70	< 0.001	0.003	< 0.001
Cys	46.0^{cd}	52.0^{bc}	48.0^{cd}	39.1^{d}	44.3^{cd}	60.5^{ab}	62.4^{a}	41.1^{d}	5.91	< 0.001	< 0.001	0.003
Glu	60.1^{d}	72.5^{c}	78.4^{bc}	85.0^{a}	63.3^{d}	76.8^{bc}	76.0^{bc}	79.1^{ab}	4.04	< 0.001	0.863	0.004
Gly	65.4^{d}	72.4^{bcd}	80.9^{ab}	89.8^{a}	65.6^{cd}	76.7^{b}	74.7^{bc}	76.7^{b}	5.66	< 0.001	0.017	< 0.001
Pro	59.6^{cd}	68.4^{bcd}	78.4^{abc}	87.5^{abc}	39.5^{d}	96.4^{ab}	101.5^{a}	91.2^{ab}	19.69	< 0.001	0.095	0.007
Ser	64.5^{c}	66.7^{bc}	70.4^{bc}	79.5^{a}	66.5^{bc}	73.0^{ab}	71.1^{b}	71.5^{b}	4.04	< 0.001	0.796	< 0.001
Tyr	64.7^{y}	72.8^{x}	74.2^{x}	79.0^{x}	67.6^{y}	77.0^{x}	77.8^{x}	77.9^{x}	4.42	< 0.001	0.039	0.340

Note: each least squares mean represents 8 observations except for pigs fed HFM and FDPP (7 observations).

[a-e]Means within a row with different superscripts differ ($P < 0.05$).

[x-z]Means within a row with different superscripts within species differ ($P < 0.05$).

CHAPTER 7. SUMMARY

7.1 Summary

An accurate determination of amino acid (**AA**) availability in feed ingredients and diets is fundamental to supply adequate quantities of AA to monogastric animals and reduce the excretion of excessive nitrogen (**N**). In both broiler chickens and pigs, availability of AA in feed ingredients has been generally evaluated as the standardized ileal digestibility (**SID**) of AA by animals fed semi-purified diets containing the feed ingredient of interest as the sole source of N (Kong and Adeola, 2014). However, imbalance or deficiency of AA in experimental diets may negatively affect the digestive physiology of animals, leading to unreliable SID values.

Similar methodology to determine the SID of AA in feed ingredients and diets has been applied to both broiler chickens and pigs (Kong and Adeola, 2014). However, broiler chickens and pigs may have different SID of AA in the same feed ingredients due to the differences in digestive organs and their capacity. Moreover, the influence of feed ingredient-specific factors which affect the SID of AA may be different between broiler chickens and pigs.

Background information for AA nutrition in monogastric animals were reviewed in chapter 1. Amino acids can be divided into indispensable and dispensable AA depending on the nutritional necessity (D'Mello, 2003). By proteolytic enzymes secreted in the foregut and small intestine, dietary proteins are digested to AA and di- and tripeptides which can be absorbed into enterocytes. Digestibility of AA has been generally determined as ileal digestibility mainly due to the microbial fermentation in the hindgut (Sauer and Ozimek, 1986). In addition, ileal digestibility of AA can be determined as apparent, standardized, or true ileal digestibility depending on the component of endogenous losses of AA used in calculations (Stein et al., 2007). Digestibility of AA can be affected by various factors including techniques used to collect ileal digesta samples, the type of indigestible index marker, the concentration of dietary fiber, antinutritional factors, and processing of feed ingredients.

The experiment in chapter 2 was designed and conducted to determine the ileal digestibility of AA in casein by regression analysis and to investigate the effects of casein in experimental diets on SID of AA in corn distillers' dried grains and solubles (**DDGS**) fed to pigs. Regression analysis between dietary and digestible AA concentrations in experimental diets revealed that the ileal

digestibility of AA in casein were close to 100%. In addition, the SID of Lys and Phe for DDGS diet containing casein were greater than the values for DDGS diet without casein. Therefore, it was concluded that the addition of protein supplements containing highly digestible AA such as casein may improve the SID of AA in feed ingredients with low quality of AA composition such as DDGS. However, it was unclear whether increased SID of Lys and Phe for DDGS diet containing casein were due to the addition of casein or due to the decreased concentration of DDGS in experimental diets.

To further investigate the relationship between composition of AA in experimental diets and SID of AA in DDGS based on the results and limitations in chapter 2, two experiments were designed and conducted in chapter 3 to determine the effects of graded concentration of casein in experimental diets on SID of AA in DDGS and to investigate the effects of DDGS concentration and casein addition to experimental diets on SID of AA in DDGS fed to pigs. The SID of most AA in DDGS linearly decreased with increasing concentration of casein in experimental diets. In addition, the SID of most AA in DDGS was reduced when the concentration of DDGS in experimental diets decreased regardless of the addition of casein.

In two experiments conducted in chapter 3, the least concentration of DDGS in experimental diets was 155.6 g/kg which provided 43.6 g/kg crude protein. Due to the reduced concentration of AA contributed from DDGS, determination of SID of AA in DDGS was susceptible to errors especially in calculations by the difference method, which may lead to decreased SID of AA in DDGS regardless of the addition of casein. Therefore, the effects of casein on SID of Lys and Phe shown in chapter 2 might not be observed in chapter 3 due to the detrimental effects of low concentrations of DDGS in experimental diets. Further research is needed to investigate the effects of graded concentrations of casein in experimental diets without reducing the concentration of DDGS. With the consideration of low concentration and digestibility of Lys in DDGS, using crystalline Lys to improve the protein quality in experimental diets may also affect the SID of AA in DDGS, which needs to be investigated further. In addition, experiments in chapter 2 and 3 used DDGS as the test ingredient with low quality AA composition; however, it remains unclear whether the addition of casein in experimental diets would increase the SID of AA in other feed ingredients. Therefore, research to verify the effects of casein on SID of AA needs to be conducted with other feed ingredients with low quality AA composition. Furthermore, potential negative effects of imbalanced or deficient AA in experimental diets can be ameliorated

not only by the addition of casein in diets but also by the parenteral provision of free AA (de Lange et al., 1989). Infusion of adequate free AA via the jugular vein of pigs during the experimental period may provide enough AA to maintain the normal physiological condition of pigs including the normal function of digestive systems in the gastrointestinal tract. Further research is required to investigate the relationship between the body AA status of pigs and SID of AA in feed ingredients when pigs are fed experimental diets containing imbalanced and insufficient AA.

The 2 experiments in chapter 4 were conducted to compare the SID of AA in full-fat soybean, soybean meal containing 430 g/kg CP, soybean meal containing 470 g/kg CP, and peanut flour between broiler chickens and pigs. Pigs had greater SID of most AA than broiler chickens, and the pattern of differences in AA digestibility among ingredients was similar between broiler chickens and pigs. Therefore, among test ingredients used in this chapter, the relative quality of AA for one species may be evaluated by those for the other species.

The objective of 2 experiments in chapter 5 was to compare the SID of AA in full-fat canola seeds, canola meal, and canola expellers between broiler chickens and pigs. Interactions were observed in SID of most AA between experimental diets and species, mainly due to the greater SID of most AA in full-fat canola seeds for broiler chickens than the values for pigs. In broiler chickens, the SID of most AA were not different among canola products. In pigs, however, the SID of most AA in full-fat canola seeds were less than canola expellers, but not different from canola meal.

Two experiments in chapter 6 compared the SID of AA in hydrolyzed feather meal, flash dried poultry protein, poultry meal, and meat and bone meal between broiler chickens and pigs. In broiler chickens, the SID of His, Thr, Trp, and Val in flash dried poultry protein and poultry meal were less than in meat and bone meal; however, there was no difference in SID of those AA among flash dried poultry protein, poultry meal, and meat and bone meal fed to pigs, which resulted in interactions between experimental diets and species. On the other hand, the SID of most AA in hydrolyzed feather meal were less than in the other test ingredients within broiler chickens and pigs.

Based on the results of the studies in chapters 4, 5, and 6, it was revealed that the effects of feed ingredient-specific factors such as fiber contents or processing on the SID of AA were different between broiler chickens and pigs. Specifically, dietary fiber in full-fat canola seeds may improve the digestive function of the gizzard in broiler chickens, but not in pigs due to the absence

of a gizzard. On the other hand, due to the greater length of the gastrointestinal tract and passage rate, pigs may have greater ability to digest and absorb AA in feed ingredients than broiler chickens which may ameliorate the negative effects of heat treatment for animal protein sources. In addition, in consideration of the results in chapter 2, the reason for the less SID of AA in full-fat canola seeds for pigs compared with broiler chickens may be related to the ratio of indispensable AA with Lys in full-fat canola seeds. Calculated ratios of indispensable AA with Lys (both total and standardized ileal digestible AA basis) in full-fat canola seeds were closer to the ideal AA patterns of broiler chickens (Baker, 2003) than those of pigs (National Research Council, 2012).

One of the limitations in the current studies was that the direct comparison of digestive capacity in the foregut or small intestine between broiler chickens and pigs was not possible. Therefore, it was unclear whether interactions between species and feed ingredients were observed due to the differences in the foregut or small intestine or other biochemical differences such as substrate specificity of proteolytic enzymes and transporters. Further research is needed to elucidate the differences between broiler chickens and pigs regarding impact of feed ingredient-specific factors on digestion and absorption of AA in the foregut and small intestine. In addition, the SID of AA may be affected by age or body weight (**BW**) of animals. Previous studies have been reported that the digestibility of nutrients and gross energy were affected by age of broiler chickens (Adeola et al., 2018) and BW of pigs (Xie et al., 2019). Moreover, basal ileal endogenous losses of AA, which is necessary for the determination of SID values, may be affected by age of broiler chickens (Adedokun et al., 2007) and BW of pigs (Park et al. 2013). Therefore, if different age or BW of animals are used to compare the SID of AA in test ingredients, results including interactions and trends may be different from the current studies. Nevertheless, results shown in the current studies may be representative because the age and BW used in the current studies were within the range which have been practically used for digestibility studies. Research to compare the SID of AA between broiler chickens and pigs needs to be conducted further with other feed ingredients which can provide the data to generate the prediction equation to estimate the SID of AA for one species using the information of the other species.

7.2 Literature Cited

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590-2597. doi:10.3382/ps.2007-00096

Adeola, O, M. N. Anwar, M. R. Abdollahi, and V. Ravindran. 2018. Age-related energy values of meat and bone meal for broiler chickens. Poult. Sci. 97:2516-2524. doi:10.3382/ps/pey100

Baker, D. H. 2003. Ideal amino acid patterns for broiler chicks. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. 2nd ed. CAB International, Cambridge, MA. p. 223-235.

de Lange, C. F. M., W. C. Sauer, and W. Souffrant. 1989. The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J. Anim. Sci. 67:755-762. doi:10.2527/jas1989.673755x

D'Mello, J. P. F. 2003. Amino acids as multifunctional molecules. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. 2nd ed. CAB International, Cambridge, MA. p. 1-14.

Kong, C., and O. Adeola. 2014. Invited review: Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

National Research Council. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186–192.

Sauer, W. C., and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367-388. doi:10.1016/0301-6226(86)90076-X

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742

Xie, F., Y. K. Li, J. B. Zhao, Z. C. Li, L. Liu, Y. H. Cao, and S. Zhang. 2019. Comparative digestibility of energy and nutrients in four fibrous ingredients fed to barrows at three different initial body weights. Can. J. Anim. Sci. 99: 315-325. doi:10.1139/cjas-2017-0094

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PUBLICATIONS

Son, A. R., C. S. Park, K. R. Park, B. G. Kim. 2019. Amino acid digestibility in plant protein sources fed to growing pigs. Asian-Australas. J. Anim. Sci. 32:1745-1752. doi:10.5713/ajas.19.0037

Park, C. S., D. Ragland, A. Helmbrecht, J. K. Htoo, and O. Adeola. 2019. Digestibility of amino acid in full-fat canola seeds, canola meal, and canola expellers fed to broiler chickens and pigs. J. Anim. Sci. 97:803-812. doi:10.1093/jas/sky470

Park, C. S., D. Ragland, and O. Adeola. 2018. Amino acid digestibility of corn distillers' dried grains with solubles with the addition of casein in pigs. J. Anim. Sci. 96:4674-4684. doi:10.1093/jas/sky309

Park, C. S., A. Helmbrecht, J. K. Htoo, and O. Adeola. 2017. Comparison of amino acid digestibility in full-fat soybean, two soybean meals, and peanut flour between broiler chickens and growing pigs. J. Anim. Sci. 95:3110-3119. doi:10.2527/jas2017.1404

Son, A. R., C. S. Park, and B. G. Kim. 2017. Determination and prediction of digestible and metabolizable energy concentrations in byproduct feed ingredients fed to growing pigs. Asian-Australas. J. Anim. Sci. 30:546-553. doi:10.5713/ajas.16.0607

Park, K. R., C. S. Park, and B. G. Kim. 2016. An enzyme complex increases in vitro dry matter digestibility of corn and wheat. SpringerPlus 5:598. doi:10.1186/s40064-016-2194-5

Park, C. S., and B. G. Kim. 2016. In vitro solubility of copper(II) sulfate and dicopper chloride trihydroxide for pig. Asian-Australas. J. Anim Sci. 29:1608-1615. doi:10.5713/ajas.16.0189

Kong, C., C. S. Park, and B. G. Kim. 2016. Evaluation of a mycotoxin adsorbent in swine diet containing barley naturally contaminated with Fusarium mycotoxins. Rev. Colomb. Cienc. Pecu. 29:169-177.

Kong, C., C. S. Park, J. Y. Ahn, and B. G. Kim. 2016. Relative bioavailability of $_{DL}$-methionine compared with $_{L}$-methionine fed to nursery pigs. Anim. Feed Sci. Technol. 215:181-185. doi:10.1016/j.anifeedsci.2016.03.011

Park, C. S., I. Park, and B. G. Kim. 2016. Effects of an enzyme cocktail on digestible and metabolizable energy concentrations in barley, corn, and wheat fed to growing pigs. Livest. Sci. 187:1-5. doi:10.1016/j.livsci.2016.02.003

Park, C. S., and B. G. Kim. 2015. Standardized ileal digestible lysine requirement of 6 to 10 kg weanling pigs. Am. J. Anim. Vet. Sci. 10:150-155. doi:10.3844/ajavsp.2015.150.155

Kong, C., C. S. Park, and B. G. Kim. 2015. Effects of enzyme complex on in vitro dry matter digestibility of feed ingredients for pigs. SpringerPlus 4:261. doi:10.1186/s40064-015-1060-1

Kong, C., S. Y. Shin, C. S. Park, and B. G. Kim. 2015. Effects of feeding barley naturally contaminated with fusarium mycotoxins on growth performance, nutrient digestibility, and blood chemistry of gilts and growth recoveries by feeding a non-contaminated diet. Asian-Australas. J. Anim. Sci. 28:662-670. doi:10.5713/ajas.14.0707

Jeong, Y. D., S. H. Lee, C. S. Park, and S. K. Park. 2015. Variation in total tract digestibility of dry matter, nitrogen, and phosphorus in different corn hybrids fed to growing-finishing pigs. Anim. Feed Sci. Technol. 201:66-71. doi:10.1016/j.anifeedsci.2015.01.002

Park, C. S., A. R. Son, D. Y. Kil, and B. G. Kim. 2015. Digestible and metabolizable energy in corn grains from different origins for growing pigs. Anim. Sci. J. 86:415-421. doi:10.1111/asj.12315

Kil, D. Y., C. S. Park, A. R. Son, S. Y. Ji, and B. G. Kim. 2014. Digestibility of crude protein and amino acids in corn grains from different origins for pigs. Anim. Feed Sci. Technol. 196:68-75. doi:10.1016/j.anifeedsci.2014.06.008

Park, C. S., S. I. Oh, and B. G. Kim. 2013. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. Rev. Colomb. Cienc. Pecu. 26:186-192.

Park, C. S., A. R. Son, and B. G. Kim. 2012. Prediction of gross energy and digestible energy in copra meal, palm kernel meal, and cassava root fed to pigs. J. Anim. Sci. 90:221-223. doi:10.2527/jas53954