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Amino acid requirements of health challenged pigs

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Amino acid requirements of health challenged pigs

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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LIST OF ABBREVIATIONS

AA = amino acid(s)
ADFI = average daily feed intake
ADG = average daily gain
AID = apparent ileal digestibility
APP = acute phase protein
ATP = adenosine triphosphate
ATTD = apparent total tract digestibility
BAAL = basal amino acid losses
BCAA = branched chain amino acid
Bhyo = *Brachyspira hyodysenteriae*
BSL2 = biosecurity level 2
BUN = blood urea nitrogen
BW = body weight
CC = composite colitis
CFA = complete Freund's adjuvant
CO_2 = carbon dioxide
CP = crude protein
Cr_2O_3 = chromic oxide
Ct = cycle threshold
DM = dry matter
DMI = dry matter intake
dpi = days post inoculation
EAA = essential amino acid
ELISA = enzyme-linked immunosorbent assay
ETEC = enterotoxigenic Escherichia coli
FCR = feed conversion ratio
GE = gross energy
GIT = gastrointestinal tract
GPx = glutathione peroxidase
G:F = gain-to-feed ratio
HCl = hydrochloride
HCW = hot carcass weight
HMB = β-hydroxy-β-methylbutyrate
HS = heat stress
HSBM = high soybean meal
ILR = irreversible loss rate
IRA = ileorectal anastomosis
ISU = Iowa State University

ISU VDL = Iowa State University Veterinary Diagnostic Laboratory
LIDIF = livestock infectious disease isolation facility
LSBM = low soybean meal
LPS = lipopolysaccharide
Lys:ME = grams SID lysine-to-megacalories metabolizable energy ratio
MD = mucoid to mucohemorrhagic diarrhea
ME = metabolizable energy
mTOR = mammalian target of rapamycin
N = nitrogen
NEAA = nonessential amino acid
NF = nitrogen free
NFD = nitrogen-free diet
NRC = National Research Council
NO = nitric oxide
OXPHOS = oxidative phosphorylation
PCR = polymerase chain reaction
PEDV = Porcine Epidemic Diarrhea Virus
PRRSV = Porcine Reproductive and Respiratory Syndrome Virus
PUN = plasma urea nitrogen
SAA = sulfur-containing amino acid
SAAL = specific amino acid loss
SBM = soybean meal
SD = swine dysentery
SOD = super oxide dismutase
SID = standardized ileal digestibility
TCA = tricarboxylic acid cycle
TID = true ileal digestibility
VTM = vitamin trace mineral

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) and *Brachyspira hyodysenteriae* (Bhyo) are two economically significant pathogenic agents in which their diseases impact pig production worldwide. Although different nutritional strategies have been utilized to ameliorate the negative impacts of disease in pigs, the mechanisms remain undefined. Interestingly, little is known about how these diseases, or several others, impact pig apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) or basal endogenous losses (BEL) of amino acids (AA), and therefore, standardized ileal digestibility (SID) values for AA have not been determined. Thus, the overall objective of this dissertation was to determine how PRRSV or Bhyo impact AID and BEL values. From these, more accurate SID values can be calculated. Hindgut nutrient disappearance in the face of these two pathogens was also determined from AID and ATTD values. Further, we evaluated the optimal lysine-to-metabolizable energy ratio (g SID Lys to metabolizable energy; Lys:ME) in PRRSV challenged pigs. To accomplish these objectives, a series of experiments were conducted and are outlined in three research chapters (Chapter 2, 3 and 4).

In Chapter 2, an experiment was conducted to assess the impact of soybean meal (SBM) and PRRSV on AID, BEL, and calculated SID values of N and AA near peak viremia (5-8 days post inoculation; dpi) and seroconversion (16-19 dpi). Similarly, in Chapter 3 an experiment was conducted to determine the impact of Bhyo on AID and BEL of N and AA, and from these SID of N and AA were calculated. The final research chapter (Chapter 4) aimed to determine the ideal dietary Lys:ME for 25 and 50 kg BW pigs challenged with PRRSV.

The results from this research indicate that the mechanism by which high SBM improves the outcome of PRRSV challenged pigs does not appear to be related to increased digestibility of N or AA as there were no interactions of SBM and PRRSV (Chapter 2). There were no reductions in ATTD of nutrients or energy from PRRSV infection; however, AID of DM and GE were reduced at 7-8 dpi only. Similarly, AID of AA were not changed due to PRRSV challenge at either collection. In contrast to PRRSV challenge, Bhyo reduced ATTD of nutrients and energy but did not change AID values outside of increasing AID of Gly (Chapter 3). Interestingly, BEL of Arg, Ala, and Pro were reduced at 7-8 dpi due to PRRSV while no BEL differences were detected at 18-19 dpi. This lead to reductions in SID of Arg, Gly, and Pro at 7-8 dpi and SID of Pro at 18-19 dpi. Only BEL of Pro was reduced due to Bhyo challenge, and when SID values were calculated, SID of N, Arg, Lys, Ala, Gly, Pro, and Ser were reduced.

When hindgut disappearance of nutrients and energy were calculated, PRRSV and Bhyo acted in an opposite manner (Chapter 2 and 3, respectively). Compared to control pigs, PRRSV increased hindgut disappearance of DM and GE at peak viremia only. In contrast, Bhyo challenge resulted in a general appearance of N and GE as opposed to control pigs that had a general disappearance. These data suggest that overall energy balance may be improved by increased energy disappearance in PRRSV challenged pigs while N and energy needs may be increased in Bhyo challenged pigs to accommodate increased losses in the hindgut.

In the final research chapter (Chapter 4), commercial pigs were utilized in an industry style production setting in which we reported that increasing the SID Lys:ME improved growth and feed efficiency in 25 kg BW and 50 kg BW PRRSV challenged pigs. Further, growth and feed efficiency were optimized at 110% to 120% Lys:ME compared to control pigs. In pigs

experimentally or naturally infected with PRRSV, the Lys:ME requirement for growth and feed efficiency was similar.

In summary of this dissertation, both systemic/respiratory (PRRSV) and colitis (Bhyo) challenges did not greatly impact ileal digestibility of nutrients, energy, and AA. Further, we did not report major changes in ileal endogenous AA losses, but hindgut disappearance of nutrients was increased and decreased in PRRSV and Bhyo challenged pigs, respectively. The AA SID values were minimally impacted by PRRSV while Bhyo reduced the SID of Arg, Lys, and some nonessential AA; however, SID values of pigs challenged with Bhyo were all above 90%. This body of work also showed that increasing dietary Lys:ME to 110% or 120% of control pig requirement was ideal for growth and feed efficiency in PRRSV challenged pigs. These results will allow pork producers and nutritionists to better formulate diets to improve performance and feed efficiency in health challenged pigs.

CHAPTER 1. LITERATURE REVIEW

Introduction

Under different physiological conditions, the dietary nitrogen (**N**), crude protein (**CP**), and amino acids (**AA**) requirements in growing pigs are determined by the metabolic demand that must be met for maintenance, protein synthesis, and/or lean tissue accretion (i.e. growth) (Escobar et al., 2004; Humphrey and Klasing, 2004). Therefore, optimal diet composition of AA, vitamins, minerals, lipids, and carbohydrates is essential to optimize energy concentrations of the diet and growth performance of growing pigs. Protein is one of the most expensive components in swine diets, second only to energy. Thus, between nutrient requirements and least cost formulating diets, efficient use of dietary AA for growth and lean tissue accretion is critical.

In classical terms, AA are considered the building blocks for proteins with 20 primary AA being incorporated into proteins (Wu, 2009). However, more than 300 natural and synthetic AA have been identified. Each of the 20 primary AA contain a unique side-chain, or *R* group, and all except proline have an amino (-NH2) and carboxyl (-COOH) group. Instead of having an amino group, proline has an imino group (-NH), making it the only proteinogenic imino acid. All AA have D- and L-isomers except glycine; however, the utilization of D-AA is generally less efficient, but this is dependent on the substrate and species (Baker, 1986; Baker, 2006).

Amino acids are often abbreviated to three letters or a single letter (Table 1.1). In the remainder of this review, AA will be referred to by their three-letter abbreviation and will be discussed in terms of essential AA (**EAA**) and nonessential AA (**NEAA**). Essential AA cannot be synthesized at a rate to meet the metabolic demands for maintenance, growth, and/or reproduction, and therefore, must be supplied by the diet. Synthesis of NEAA is therefore

assumed to be at a level sufficient to support normal physiological function. During some periods of growth or in disease states, utilization rates of some NEAA are increased above what the pig synthesizes and are considered conditionally essential. These include Arg and Pro in newly weaned pigs to maximize protein synthesis (Ball et al., 1986), and Cys, Tyr, and Glu can become essential during weaning or disease stress to support immune and antioxidant function (Rezaei et al., 2013).

Table 1.1. Essential, conditionally essential, and nonessential amino acids.

Essential	Conditionally essential	Nonessential
Histidine, His, H	Arginine, Arg, R	Alanine, Ala, A
Isoleucine, Ile, I	Cysteine, Cys, C	Asparagine, Asn, N
Leucine, Leu, L	Glutamine, Gln, Q	Aspartate, Asp, D
Lysine, Lys, K	Proline, Pro, P	Glutamate, Glu, E
Methionine, Met, M	Tyrosine, Tyr, Y	Glycine, Gly, G
Phenylalanine, Phe, F		Serine, Ser, S
Threonine, Thr, T		
Tryptophan, Trp, W		
Valine, Val, V		

Shown as full name, three-letter abbreviation, and one-letter abbreviation
Adapted from (NRC, 2012)

Amino acids are primarily thought of as the precursors or substrates for protein synthesis but are involved in several other physiological functions (Li et al., 2007; Wu, 2009). For example, Lys, Gln, and Asp are precursors for purine and pyrimidine bases used in DNA and RNA synthesis. Others, such as Arg and Gln, are precursors for non-protein AA such as ornithine and citrulline that play a role in the urea cycle. Amino acids can also be utilized as energy sources, serving as precursors for glucose or ketones. Although less efficient than glucose or fatty acids, AA can be completely oxidized for ATP production to provide energy when there is an energy deficiency (Wu, 2009).

Although all 20 proteinogenic AA are essential for efficient growth in pigs, research has primarily focused on Lys, Met, Thr, and Trp, commonly the first four limiting AA in healthy pig diets; however, the order of limiting AA may be altered in health challenged pigs. For example, based on their incorporation into acute phase proteins (**APP**), the first limiting AA are Phe, Trp, and Ser, respectively, while Met, Thr, and Lys are the 7, 8, and 17th AA incorporated into APP, respectively (Reeds et al., 1994). This would suggest that AA priorities and requirements are different for healthy and health challenged pigs. Therefore, this literature review is divided into two main sections. The first part of this review will examine methods to determine AA requirements, digestibility, and endogenous losses of AA. The second section of the review will detail the significance of AA and CP nutrition and metabolism in health compromised pigs.

Estimation of Amino Acid Requirements

Nitrogen requirements include what is needed for protein synthesis, such as AA and the production of other nitrogenous compounds like urea and glutathione. Amino acid requirements refer to the amount of AA necessary to fulfill the requirement for maintenance and the requirement for growth or protein tissue accretion. Although skeletal muscle serves as a reservoir for AA (i.e., protein stores), pigs also have blood and cytosolic pools of AA that are tightly regulated. If EAA intake is limited via the diet, this can lead to deficiencies in EAA in both the free pool and tissues. Thus, the first limiting AA is the AA that most closely matches, or limits, a metabolic need (Reeds and Jahoor, 2001). By definition a limiting AA is essential, and if one AA is limiting, the other AA are not. Ingredients that make up the bulk of practical U.S. swine diets, such as corn and soybean meal, are deficient or first limiting in Lys, Met, Trp, and Thr (Table 1.2). Therefore, the bulk of AA requirement research has focused on EAA and less on NEAA

and N requirements. Because crystalline AA have become more available and affordable, low CP diets can be fed while maintaining growth performance. This reduces N excretion into the environment; however, NEAA synthesis requires a N source, therefore, when N is limited EAA are used to meet requirements of NEAA production.

With regard to domestic pig nutrition, the current Nutrient Requirements of Swine (NRC, 2012) summarizes empirical studies evaluating AA needs of growing pigs to recommend dietary AA requirements of pigs at different life cycle stages. Although several studies have focused on the limiting AA (i.e., Lys, Met, Thr, and Trp), requirements of AA that are typically in excess in practical diets (i.e., Leu and Arg) are less defined. Similarly, AA requirements in the NRC reflect those of healthy pigs, and not necessarily those of animals undergoing health or environmental challenges.

Table 1.2. Limiting amino acids in common ingredients, diets, and by pig body weight. Adapted from Neutkens, 2005.

	Limiting Amino Acids			
	1st	2nd	3rd	4th
Ingredients				
Corn	Lys	Trp	Thr	Ile
Wheat	Lys	Thr	Ile	Val
Barley	Lys	Thr	M+C	Ile
Soybean meal	M+C	Thr	Lys	Val
Canola meal	Lys	Thr	Trp	Ile
Fish meal	Trp	Thr	M+C	Val
Dried plasma	Ile	M+C	Lys	Thr
Dried whey	M+C	Lys	Val	Trp
Diets				
Corn-soybean meal	Lys	Thr	Trp	M+C
Wheat-soybean meal	Lys	Thr	Ile	Val
Corn-soy + fish meal	Lys	Trp	Thr	M+C
Corn-soy + dried whey[b]	M+C	Lys	Thr	Trp
Corn-soy + whey + plasma[b]	M+C	Thr	Trp	Val
Body weight (corn-soy diet)				
10 kg	Lys	M+C	Thr	Trp
20 kg	Lys	Thr	M+C	Trp
50 kg	Lys	Thr	Trp	M+C

[a]Based on NRC (2012) ingredient composition and amino acid requirements for 50 kg pig
[b]Based on a 10 kg BW pig requirement
M+C = methionine + cysteine

Amino acid requirements can be determined using direct (empirical) or indirect methods. Empirical experiments consist primarily of dose-response, or titration, experiments. For examples, various amounts of an AA of interest are fed, and a collection of response criteria are measured. These criteria are often related to production traits such as ADG, body composition, protein accretion, or feed efficiency in growing animals, and milk yield and litter size in reproducing and lactating animals. For the bulk of this review, AA requirements and methodology will focus on production traits of growing pigs.

Requirements for different production traits can warrant different AA levels. For example, AA requirements for improved feed efficiency tend to be higher than those for BW gain (NRC, 2012). Similarly, AA requirements can be influenced by endogenous factors like breed and genotype (Schneider et al., 2010; Liu et al., 2015), sex (Cromwell et al., 1993), and age (Martinez and Knabe, 1990), as well as exogenous factors such as dietary composition (Baker et al., 1975; Webster et al., 2007) or environmental and immunological stressors (Han and Baker, 1993; van Heugten et al., 1994; Rakhshandeh et al., 2014).

Empirical studies to determine AA requirements utilize a range of AA amounts from deficient to excessive. Formulating a diet that is deficient in a single AA while remaining adequate in others can be challenging using least-cost formulation and commercially applicable ingredients. To ensure a diet is deficient in AA, a purified, protein-free diet based on cornstarch can be fed; however, palatability can be impacted when using cornstarch-based diets (Otto et al., 2003). A more common approach is formulating a basal diet using ingredients that are low in the test AA and supplementing the diet with crystalline AA to ensure that the test AA is the first limiting (Litvak et al., 2013b). Difficulties can arise from disproportionate amounts of AA in the basal diet. Consequently, the response could be a result of AA imbalance or antagonism instead

of the first limiting AA. Although expensive, an alternative method is using a highly digestible AA source like casein, with digestibility assumed to be near 100% (Chung and Baker, 1992a).

Other ways to assess AA requirements in growing pigs can include N balance (Brown et al., 1974), plasma AA concentrations (Mitchell et al., 1968), and AA oxidation (Ball et al., 1986). Although production traits are not the primary response criteria for these dose titration studies, AA requirements can be determined using metabolic responses such as AA flux, disappearance, or retention which are more tightly regulated and therefore less variable.

Nitrogen Balance

Nitrogen balance or retention is a classic method for determining protein and thus AA nutrition. Nitrogen retention increases with the dietary addition of a limiting AA that allows for AA requirements and efficiency to be determined (Thong and Liebert, 2004). This methodology is relatively simple, inexpensive, and does not require invasive surgeries or techniques that could alter AA metabolism. Simply, this method determines the difference between intake (feed consumption input) and excretion (feces and urine output) of N. A dose-response curve can then be constructed, and the requirement is estimated to be where N equilibrium between output and input is met (Fig. 1.1).

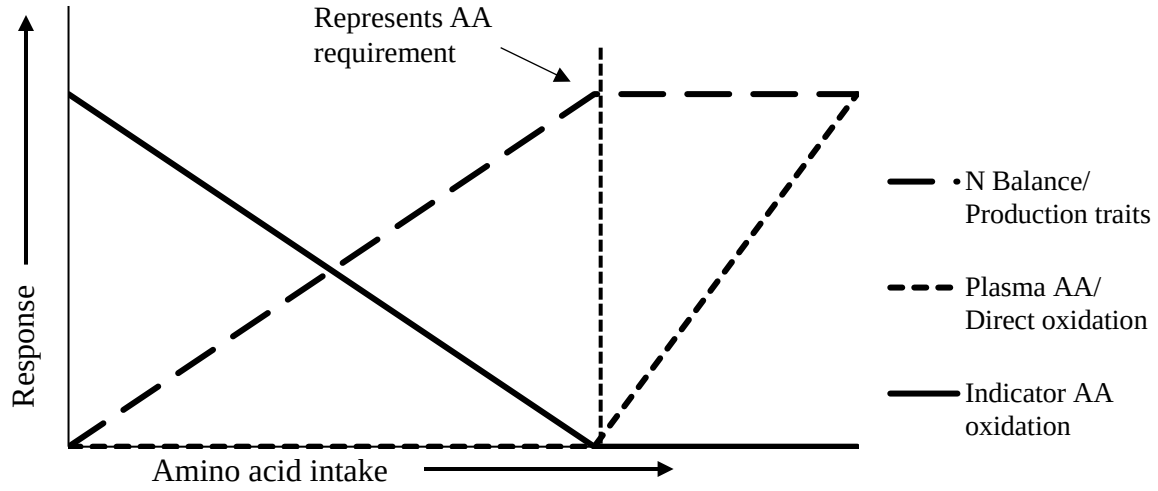

Figure 1.1. Patterns of response to graded amino acid intakes using different evaluation methods. Adapted from Pencharz and Ball, 2003.

Plasma Amino Acid Kinetics

Although more technical and expensive, catheterization of the portal vein and carotid artery along with a blood flow probe can be used to determine AA absorption and kinetics in pigs (Rerat et al., 1980; Hooda et al., 2009). The absorption of specific AA can be determined by the difference in portal and arterial differences once adjusted for blood flow through the portal vein. In this method, nutrient and AA utilization by intestinal enterocytes (first-pass metabolism) can be accounted for; however, it does not allow for the contribution (addition or subtraction) of AA by the intestine tract to the measured AA pool. This method is beneficial as it allows for chronological estimation of nutrient appearance in the blood and produces similar AA digestibility coefficients to other methods (Rerat et al., 1980).

Tracer and Indicator Amino Acid Method

A tracer method using radiolabeled AA to determine AA oxidation can determine AA requirements (Chavez and Bayley, 1976; Brunton et al., 2007). When an AA is limiting, a significant portion is used for protein synthesis, and little is oxidized to CO_2. Increasing the AA above requirement results in increased oxidation of that AA to CO_2. When a radiolabeled AA is intravenously infused in addition to graded AA levels in the diet, the release of radioactive CO_2 can be measured. When an AA cannot be efficiently labeled, the oxidation of an indicator AA can be determined (Kim et al., 1983). The basis for an indicator AA is that as the supply of a limiting AA increases, the oxidation of other non-limiting AA decreases until the requirement for the limiting AA is met. This allows for the irreversible loss rate (**ILR**) of AA to be calculated, which reflects the amount of free AA that disappear from the plasma pool per unit of time for protein synthesis and oxidation.

Once AA requirements have been determined, they can be expressed in several different ways. Requirements can be expressed as the total amount in the diet. Requirements are generally expressed as a percent of the diet in *ad libitum* fed pigs but can also be expressed on a grams/day basis when feed may be restricted. Additionally, because pigs eat to meet their energy needs (Nyachoti et al., 2004) when dietary energy density or physiological factors (i.e., disease) impact feed intake, AA requirements can be expressed relative to dietary energy. A final approach to presenting AA requirements is relative to other AA, usually the first limiting AA principles. This method gives the optimal pattern of AA in relation to one another and is referred to as the “ideal protein” concept.

Ideal Protein Concept

Mitchell (1962) first conceptualized ideal protein more than 50 years ago. The concept has been advanced by Fuller and Baker in livestock, primarily poultry and swine. This concept refers to all EAA being co-limiting for performance, so the AA supply matches AA requirement as exactly as possible. In healthy pigs Lys is the first limiting AA for growth, therefore AA in pig diets are displayed as a ratio to Lys (Lys = 100%). In an ideal protein model, requirements are set at a tissue requirement, generally for support of maximum protein deposition. Although Lys requirement changes with age, it is assumed that the ideal protein profile and AA ratio to Lys do not. Therefore, each ratio relative to Lys remains constant throughout the life cycle of the pig (van Milgen and Dourmad, 2015).

Initially, Cole (1980) formulated the dietary EAA profile to match the pig carcass EAA profile. This initial concept was imperfect, however, because dietary AA can be synthesized into other AA (ex. Arg synthesis from Pro) in the small intestine, circulating AA and dietary (luminal) AA concentrations differ, plasma AA have different metabolic fates in different tissues, and tissue AA abundance differs from AA abundance in the diet (Bertolo et al., 2003; Wu, 2014). The initial concept did, however, include a N requirement to support NEAA synthesis (Cole, 1980). Wang and Fuller (1989) and Fuller et al. (1989) improved the original concept by estimating AA requirements for maintenance and protein accretion. This was then improved upon again by Chung and Baker (1992b) who also considering some NEAA. Wang and Fuller (1989) also determined that there is an optimum EAA:NEAA ratio of 45:55 equating to 0.42 EAA:total N in 25-45 kg BW pigs. Heger et al. (1998) further verified EAA:total N and determined the optimum EAA:total N to be 0.60 to 0.66 in 45 kg BW pigs. The ideal protein

profile varies between institutions due to the methods used to determine requirements (Table 1.3).

Table 1.3. Ideal protein profile in different institutions

Ratio	NRC (2012) USA	INRA (2013) France	VSP (2013) Denmark
Lys:Lys	100	100	100
Thr:Lys	59	65	61
Met:Lys	29	30	32
(Met+Cys):Lys	55	60	54
Trp:Lys	16	22	20-22
Val:Lys	63	70	67
Ile:Lys	51	52	53
Leu:Lys	100	101	102
His:Lys	34	31	32
Phe:Lys	58	54	57
(Phe+Tyr):Lys	93	-	111
Tyr:Lys	-	40	-

Ratios are indicative of standardized ileal digestibility values
Adapted from NRC (2012), van Milgen and Dourmad (2015), VSP (2013)

It is conceivable that the ideal protein profile is altered in times of disease or immune stimulation. Thus, the ideal protein profiles would likely vary with the type (enteric, respiratory, systemic) and severity of disease. However, many of these factors have not been elucidated. In most disease states, there may need to be a correction for reduced feed intake, therefore, it may be better to express AA requirements relative to dietary energy (Lewis, 2002). Regardless of the currency used to describe AA requirements (relative to Lys or energy), digestion, absorption, and transport of AA to the target tissue must occur before protein synthesis can take place, leaving multiple steps for catabolism to occur. This leads to a potential discrepancy between AA intake and AA demand. To account for these digestibility differences, values are corrected or standardized, to reflect a more accurate AA availability for protein synthesis.

Amino Acid Digestibility

One method the NRC (2012) reports AA requirements are on a total basis (grams/day); however, a portion of dietary AA is unable to be utilized by the pig. Heat treatment of some feedstuffs can damage AA through Maillard reactions and render some AA less bioavailable (Hurrell and Finot, 1983). A Maillard product is the result of the reaction between a reducing sugar and AA, particularly Lys, altering the nutritional value of that AA (Rerat et al., 2002). Therefore, it is essential to understand the digestibility and bioavailability of AA in addition to dietary intake. In general, nutrient digestibility is determined using the direct, difference, or regression method. Although digestibility of some nutrients can be determined in the feces, AA digestibility is more commonly determined at the terminal ileum. This is because hindgut microbes can utilize protein and AA that escape digestion in the small intestine (Mason et al., 1975; Torrallardona et al., 2003), and AA absorption is limited in the cecum and colon (Rérat, 1978). Thus, total tract digestibility of AA is not synonymous with AA absorption, and it is, therefore, more accurate to determine AA digestibility at the terminal ileum.

Direct, Difference, and Regression Method

The direct method uses a diet formulated so the test ingredient is the sole dietary source of the AA in question (Lin et al., 1987). In this method, AA digestibility in the test ingredient corresponds with the value in a test diet. This method is used for palatable ingredients, like many of the cereal grains used for pig feed. The direct method can also be applied to the use of semi-purified diets, typically based on cornstarch, because the test ingredient still provides the only dietary AA. When the test ingredient cannot supply adequate amounts of the AA in question, the difference and regression methods are used.

The difference method utilizes a basal and trial diet. This procedure determines the digestibility of the basal diet components in one group of pigs. Another group is fed a test diet with a known amount of basal diet replacing the test ingredient. It is common for the test diet to be formulated as the basal diet plus a given amount of the test ingredient. The digestibility of the test ingredient component can then be calculated as described by Kong and Adeola (2014) with known digestibilities of the basal diet, test diet, and test ingredient and the amount of component contributed by the test ingredient in the test diet. The difference method assumes there is no interaction between digestibility values in the basal and test diets. This method is commonly used for ingredients that are less palatable (ex. blood meal) or have a high concentration of anti-nutritional factors.

The regression method was first described in ruminants (Giger and Sauvant, 1983); however, this model has been used in pigs and poultry to determine digestibility and endogenous loss of CP and AA (Adeola et al., 2016). This method is beneficial in that basal and trial feed ingredients can be evaluated simultaneously. The basal and test ingredients can be mixed at various graded levels and allows for multiple test diets to be evaluated. Similar to the difference method, this method assumes there is no interaction between digestibility values in the basal and test ingredients. If no interaction exists, the relationship between digestibility values in the test diet and contribution levels of the AA from the basal and test ingredients can be derived by fitting the digestibility coefficients to a linear regression model. This data can then be extrapolated to 100% replacement to determine the digestibility of a component in experimental diets (Adeola, 2001).

Collection Methods and Techniques

Digestibility can be determined using either a total collection or index method. In both methods, pigs must adapt to the diet for at least 5 days (Agudelo et al., 2010), and feeding level is slightly reduced (90% *ad libitum*) to reduce feed refusal. After diet adaptation, total ileal digesta are collected for 2 to 5 days in a total collection method. Collections are performed using the marker-to-marker method where a colored, indigestible compound is fed at the beginning and end of the collection period. Commonly used markers are ferric oxide, chromic oxide, and indigo carmine. The marker must move with the digesta in the intestinal tract and cannot diffuse to unmarked digesta (Adeola, 2001). Digesta is then collected at the start and end of observation of marker-color in the digesta. Total collections are difficult to perform because it is challenging to collect all outputs. Even so, techniques are available for total collection of ileal digesta; however, all require surgical procedures on the pig (Fig. 1.2).

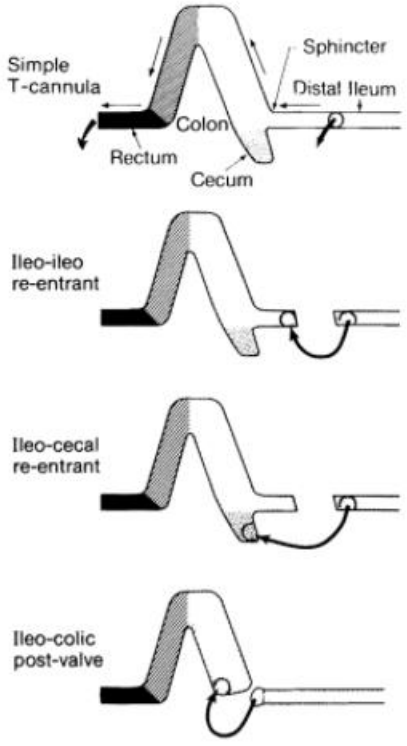

Figure 1.2. Schematic representation of cannulation techniques for the collection of ileal digesta (Sauer and de Lange, 2002)

The most straightforward collection technique is the slaughter method (Low, 1977), where pigs are euthanized, dissected, and ileal contents are collected for analysis. This procedure is not commonly used due to the large number of animals required and the animal-to-animal variation because observations can only be taken at one time point

from each animal. Also, sloughing of intestinal cells during slaughter can influence the digestibility of N and AA making the predictions less accurate (Badawy et al., 1957). To avoid cell sloughing during euthanasia, pigs can be anesthetized or euthanized with barbiturate overdose (Badawy, 1964). When compared with the T-cannula technique, apparent digestibility values were similar for this slaughter-euthanasia method (Donkoh et al., 1994).

The most common method for determining ileal AA digestibility is the index method paired with a simple T-cannula placed in the terminal ileum. The index method utilizes an indigestible marker that is totally indigestible, nontoxic to the animal, can pass through the digestive tract at an even rate and is uniformly distributed in the digesta, and is easily analyzed (Moughan et al., 1991). Surgically implanting a simple T-cannula in the distal ileum is considered less invasive as it avoids removing parts of the intestinal tract (Fig. 1.2). A shortcoming to using a T-cannula is that only a portion of the ileal digesta outflow is collected. Therefore, attention needs to be paid to frequency and duration of sampling in relation to feeding time and frequency. Also, because not all digesta is collected an indigestible marker must be used. Chromic oxide and titanium dioxide are the most commonly used markers, added in the diet from 0.1% to 0.5% (Jagger et al., 1992; Kerr et al., 2010).

Total collection methods include a re-entrant cannula that diverts the flow of digesta outside the body where digesta can be collected, then returns to either the ileum (ileo-ileo re-entrant cannula) or cecum (ileo-cecal re-entrant cannula) (Sauer and de Lange, 2002). The ileorectal anastomosis (**IRA**) technique used by Fuller and Livingstone (1982) also allows for total collection. This technique fits the ileum as an end-to-side anastomosis to the rectum which removes the hindgut from the digestive tract and allows for digesta to be collected at the anus. The IRA is advantageous to the re-entrant cannula because it requires less cannula maintenance

and feed intake remains at normal levels; however, volatile fatty acid concentrations are increased in IRA pigs suggesting the digestive tract adapts to loss of a functional large intestine (Köhler et al., 1992).

Newer methods include post-valve T-cecum cannulation and steered ileo-cecal valve cannulation. Post-valve T-cecum cannulation involves the insertion of a T-cannula into the cecum with a valve to allow for collection or for digesta to flow normally through the hindgut (van Leeuwen et al., 1988). Steered ileo-cecal valve cannulation involves the insertion of a cannula into the large intestine (Mroz et al., 1996). A metal ring, with an attached nylon cord, is secured in the ileum. During collection periods, the cord is pulled to allow for ileal digesta to exit the cannula. When the cord is released digesta flows normally through the hindgut. All these techniques give reliable digestibility data; however, they impact normal physiological digestive and absorptive processes, require complex and expensive surgery, and risk cannular blockage which makes them less desirable methods to determine AA digestibility.

Apparent and Standardized Ileal Digestibility

After nutrient concentrations and indigestible marker concentrations, if using the index method, in the diet, digesta, and feces have been analyzed, the apparent ileal (**AID**) digestibility and/or apparent total tract digestibility (**ATTD**) can be calculated. The total collection method is calculated using equation [1] and index method using equation [2] (Stein et al., 2007; Adeola et al., 2016):

$$\text{AID, ATTD (\%)} = [(AA_{intake} - ilealAA_{output}) \div AA_{intake}] \times 100 \qquad \text{[Equation 1]}$$

$$\text{AID, ATTD (\%)} = [1 - (AA_{digesta} \div AA_{diet}) \times (M_{diet} \div M_{digesta})] \times 100 \qquad \text{[Equation 2]}$$

where $AA_{digesta}$ and AA_{diet} represent the DM AA concentrations (g/kg) in digesta and diet, respectively, and M_{diet} and $M_{digesta}$ represent the DM marker concentrations (g/kg) in diet and digesta, respectively.

Apparent ileal digestibility of AA is calculated based on the percent of AA that do not appear in digesta. Although using AID values will improve the accuracy of diet formulation, they do not differentiate between dietary and endogenous AA sources recovered at the distal ileum.

To reduce variation seen with AID values and more accurately predict digestibility, AID values can be adjusted for basal (**BAAL**) or specific (**SAAL**) AA losses. These losses will be discussed in a later section.

The most common adjusted digestibility measure is standardized ileal digestibility (**SID**). This accounts for BAAL and not SAAL, and can be calculated according to equation [3] or [4], respectively, if AID values have already been calculated (Stein et al., 2007):

$$\text{SID (\%)} = \{[AA_{intake} - (\text{ileal AA outflow} - \text{BAAL})] \div AA_{intake}\} \times 100 \qquad \text{[Equation 3]}$$

$$\text{SID (\%)} = \text{AID} + [(\text{BAAL} \div AA_{diet}) \times 100] \qquad \text{[Equation 4]}$$

Standardized ileal digestibility is the intermediate between AID and true ileal digestibility (**TID**). In practical feed formulation using SID can overcome some of the limitations of AID and TID. Compared with AID, SID values are more likely to be additive in mixed diets (Stein et al., 2005), and most common feed ingredients have a SID estimate (NRC, 2012). Therefore, AA requirements are more accurately reported as SID compared to AID or total basis.

True ileal digestibility is the portion of dietary AA that disappear from the gut prior to reaching the distal ileum. Only undigested dietary AA in the ileal AA outflow are related to AA intake, not BAAL (Stein et al., 2007). True ileal digestibility AA requirements vary based on diet composition due to SAAL induced by the diet. This method is rarely used because data are hard to obtain, consequently, there is insufficient information for many feed ingredients.

Endogenous Losses

To further improve AA utilization by the pig and to optimize diet formulation, studies can be performed to determine the endogenous AA losses. Endogenous AA losses are determined from the AA profile of endogenously synthesized proteins secreted into the lumen which have not been digested and reabsorbed (Stein et al., 2007). Endogenous N and AA sources can be digestive enzymes, muco-proteins, sloughed cells, ingested hair, peptides, and amines (Moughan et al., 1992a), and can be broken into basal and specific losses (Fig. 1.3). These endogenous losses represent N and AA that cannot be captured or recaptured by the digestive tract for growth or maintenance uses by the pig.

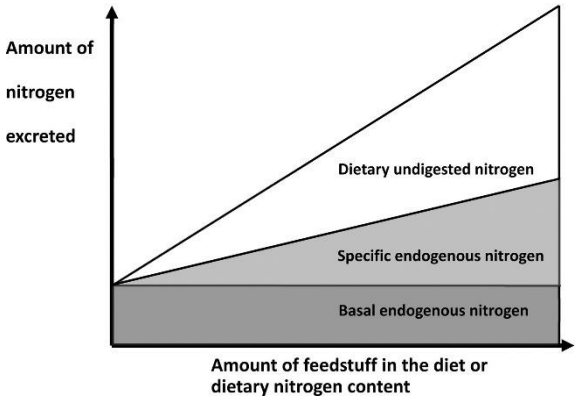

Figure 1.3. Partitioning of ileal nitrogen flow. (Adeola et al., 2016).

Basal endogenous AA losses represent the quantity of AA inevitably lost by the pig and are related to the physical flow of feed through the digestive tract or the metabolic state of the animal (Stein et al., 2007). These losses are not influenced by dietary composition and decrease with increasing DMI (Furuya and Kaji, 1992; Moter and Stein, 2004). Furthermore, Hess and

Seve (1999) demonstrated that BAAL per kg DMI decrease with increasing BW, therefore, BAAL should be established when animals are fed close to *ad libitum* and expressed in relation to DMI (Boisen and Moughan, 1996; Jansman et al., 2002).

Specific endogenous losses are losses above BAAL that are influenced by specific dietary factors such as protein level, fiber type, and anti-nutritional factors (Stein et al., 2007). High dietary CP can increase SAAL due to increased digestive enzyme secretion such as pancreatic proteases (Nyachoti et al., 1997a; Nyachoti et al., 2000; Eklund et al., 2008). Similarly, neutral detergent fiber inclusion can impact digesta viscosity and passage rate, which can affect mucin secretion thereby impacting specific losses (Mariscal-Landin et al., 1995; Mariscal-Landín et al., 2017). Anti-nutritional factors, such as trypsin inhibitors, in dietary ingredients can increase SAAL (Barth et al., 1993).

Methods for Determination of Endogenous Amino Acid Loss

Methods for determining BAAL have been developed in the last few decades. Conventional methods include feeding a nitrogen-free diet (**NFD**), feeding highly digestible protein or enzyme hydrolyzed diets, ^{15}N isotope technique, and mathematic regression methods. These methods and their advantages and disadvantages are briefly discussed.

Nitrogen-free diet method

Feeding a NFD is the most common method of determining BAAL. Because there is no protein in the diet, all N-containing compounds recovered from ileal digesta are assumed to be endogenous. The primary concern of this method is its non-physiologic nature (Low, 1980) that

can impact normal body protein metabolism and reduce gut secretion and reabsorption of endogenous N sources (Darragh et al., 1990; Nyachoti et al., 1997b). Although animals are in a negative protein balance, endogenous loss of EAA are not majorly affected (de Lange et al., 1989). Some NEAA, namely Pro and Gly, are generally overestimated (Moughan et al., 1992b), and BAAL of Pro can be increased when NFD are fed for extended periods of time (Jansman et al., 2002). The NFD method may lead to an underestimation of BAAL due to a lack of endogenous enzyme secretion because there is no dietary protein (Butts et al., 1993). As previously mentioned, dietary factors, such as fiber and anti-nutritional factors, can enhance BAAL. If a NFD is used, BAAL is typically measured using an indigestible marker according to Eq. [5]:

$$BAAL = AA_{digesta} \times (M_{diet} \div M_{digesta}) \qquad \text{[Equation 5]}$$

where BAAL is the basal endogenous loss of an AA, $AA_{digesta}$ is the AA concentration in the ileal digesta, and M_{diet} and $M_{digesta}$ are the concentrations of the indigestible marker in diet and digesta, respectively. All concentrations are reported in g/kg DMI.

Highly digestible or enzymatically hydrolyzed protein

An alternative to the NFD method is feeding a diet with a protein source that is assumed to be 100% digestible. Casein is commonly used as a protein source; however, the true digestibility of casein should be tested prior to each experiment. Using this method has provided mixed results when compared to the NFD method. Golian et al. (2008) reported similar BAAL values between methods while Fuller and Cadenhead (1991) reported lower BAAL in the casein supplemented diet compared to the NFD. de Lange et al. (1989) suggests that lower BAAL in

casein supplemented diets is associated with positive protein balance compared to negative protein balance in NFD.

Stable isotope technique

An alternative method for determining BAAL include the use of a stable isotope. Isotope dilution using ^{15}N has been used to label the N pool in the animal (Leterme et al., 1998) or dietary N (Roos et al., 1994). This allows for the differentiation between endogenous N and undigested N from the diet. It is less common to feed ^{15}N as it can undergo transamination in the gut and spread to other AA, therefore, it is more common for ^{15}N labeled AA to be continuously infused intravenously. The labeled AA is measured in the ileal digesta relative to the precursor pool (deproteinized fraction of plasma) for endogenous protein synthesis. This technique is criticized for the difficulty to attain a steady state and choosing the correct precursor pool (Moughan et al., 1992a; Leterme et al., 1998). Also, endogenous N loss can be underestimated by not accounting for endogenous mucosal cells that are synthesized using the labeled luminal AA and re-secreted (Roos et al., 1994).

Regression method

The regression method explained in the direct, difference, and regression method section can also be applied to the determination of BAAL. Graded protein levels are fed, and N or AA recovery is determined and related to N or AA intake. Extrapolation equations can determine N or AA recovery when there is no dietary N and AA intake can be estimated. This method may yield better estimates compared to NFD (Fan et al., 1995); however, estimates may not be different from those in NFD studies (Fan and Sauer, 1995; Mosenthin et al., 2007).

Amino Acid Requirements of Health Challenged Pigs

AA Utilization in Health Challenged Pigs

Amino acid requirements and utilization may vary in growing pigs depending on the immunological, inflammatory, or pathogenic insult and severity of the insult they are experiencing. A summary of the published literature investigating the impact of immunological challenges as they relate to CP and AA nutrition in pigs is presented in Table 1.4. A search of the literature returned 55 peer-reviewed articles, 2 theses, and 1 National Pork Board report that have researched CP and/or AA in health challenged pigs. From these 58 documents, approximately half of these papers used a live pathogen challenge model (n=33), while the other 43% used an adjuvant to model an immune or inflammatory response (n=25). Furthermore, much of the reported research focused on an acute time response (2-5 days post inoculation, **dpi**) using live enteric pathogens or unsanitary environmental challenge conditions (Table 1.4).

Many of the studies involving enteric pathogens could be divided into two periods: 1) a pre-challenge and 2) post-challenge period, with the parameter(s) of interest compared across both periods. Although changes in AA metabolism can be defined using the methodological approach outlined above, these enteric pathogen studies often lack an age-matched control cohort to directly compare against (i.e., a negative control treatment group). Further, many of these studies used nursery pigs immediately or very shortly after weaning. Weaning is a time of reduced growth and feed intake, compromised digestibility, and increased stress and disease susceptibility, and all of these factors improve as the pig ages (Wolter et al., 2003; Boudry et al., 2004; Moeser et al., 2007). Therefore, growth data in the pre-challenge (control) period of these studies may be confounded by the effects of weaning, providing a likely reason why growth performance is not different between pre- and post-challenge periods in many enteric pathogen

studies. Although growth data may be confounded, pathogens such as *Escherichia coli*, *Salmonella* Typhimurium, porcine epidemic diarrhea virus (**PEDV**), porcine reproductive and respiratory syndrome virus (**PRRSV**), and vaccine administration are most common in weanling pigs (USDA, 2015). Therefore, it is also important to match pig age with the pathogenic agent they would most commonly encounter at that particular stage of production. Thus, it is reasonable that many of the reported enteric pathogen studies have used pathogenic *Escherichia coli* around weaning and in early nursery age pigs.

Enteric bacteria

Interestingly, N and AA digestibility in pigs exposed to a live enteric bacterial pathogen has not been extensively studied. In 7 kg BW pigs, enterotoxigenic *Escherichia coli* (**ETEC**) challenge reduced AID of N and all AA at 7 dpi; however, by 14 dpi no differences were noticed (Heo et al., 2010b). It was also noted that a decrease in CP might improve intestinal health by reducing proteolytic fermentation in the hindgut in 5 and 7 kg BW pigs (Opapeju et al., 2009; Heo et al., 2010a; Heo et al., 2010b), as well as reducing ETEC counts and increasing butyrate-producing bacteria in 5 kg BW pigs (Opapeju et al., 2009). Although only used in a limited number of studies, *Salmonella* Typhimurium decreased AID coefficients while increasing BAAL of many AA. This resulted in an acute (24-h) increase in SID of His and Gly, while Lys SID was reduced at 72-h (Lee, 2012).

Collectively, 6 to 8 kg BW pigs exposed to enteric bacterial pathogens may have an increased requirement of Trp and Thr for growth or feed efficiency (Capozzalo et al., 2012; Ren et al., 2014; Capozzalo et al., 2015; Capozzalo et al., 2017), and potentially increased SAA although reports are mixed (Kahindi, 2014; Capozzalo et al., 2017). Similarly, in 7 kg BW pigs

challenged with attenuated *Salmonella* Cholerasuis, increasing dietary Arg improved growth and feed intake (Chen et al., 2012).

Unsanitary environments

Responses gained during periods where pigs were raised in unsanitary environments may provide a more realistic interpretation of AA requirements and metabolism differences for pigs raised in commercial production settings. These studies encompassed a broader range of BW (7 to 112 kg BW) compared with enteric challenges and typically lasted for longer durations (21 to over 100 days) allowing for a more accurate interpretation of the longitudinal impact of immune stress. Compared to pigs raised in clean sanitized environments (i.e., power washed and disinfected housing), pigs in unsanitary environmental conditions had decreases in growth (Williams et al., 1997a, b, c; Le Floc'h et al., 2009; Le Floc'h et al., 2010; Kahindi et al., 2013; Jayaraman et al., 2015; van der Meer et al., 2016; Jayaraman et al., 2017a), protein deposition (Williams et al., 1997b, c) and N digestibility (Williams et al., 1997a; Kampman-van de Hoek et al., 2016; van der Meer et al., 2016) by 11-25%, 20-25%, and 1-5%, respectively. Interestingly, data attempting to determine AA requirements was highly variable. Williams et al. (1997b) and Kahindi et al. (2013) reported increased performance, protein accretion, and feed efficiency with increased dietary Lys which is in agreement with Kampman-van de Hoek et al. (2016) who reported increased ILR of Lys; however, Williams et al. (1997a, 1997c) also reported a decreased Lys requirement for growth due to a decreased ability for protein accretion. Similar study designs have also reported no performance gains from varying levels of Thr (Jayaraman et al., 2015), Trp (Le Floc'h et al., 2010; Jayaraman et al., 2017a), or SAA (Kahindi, 2014). These differences and high variability between studies in the unsanitary environments may be a result

of differences in environmental pathogen burdens, vaccine history of pigs, age, and seasonal effects. Surprisingly, the pathogenic burden of the pigs housed in unsanitary conditions was not well characterized in all studies and diets utilized often did not report antibiotic inclusions or use.

Viral challenges

The extent to which viruses also impact AA nutrition and requirements is less known than bacteria. Only 2 studies reported AA digestibility with PRRSV, PEDV, or a combination of PRRSV and PEDV (Rakhshandeh, 2015; Schweer et al., 2016b). In a non-peer-reviewed short research report, infection with PRRSV decreased AID of N in 9 kg BW pigs (Rakhshandeh, 2015); although Schweer et al. (2016b) reported no differences in ATTD and AID of N or AID of AA due to PRRSV in 16 kg BW pigs. These discrepancies may be pig BW, age, or viral pathogenicity dependent. Rakhshandeh (2015) also reported a decreased N balance and increased utilization of Met and Thr based on ILR. Similarly, increased dietary Thr improves the immune response and N balance of pigs vaccinated with live Pseudorabies virus (Mao et al., 2014) or attenuated classical swine fever virus (Defa et al., 1999).

Inflammatory and oxidative stress agents

Although live pathogens are ideal for determining their impact on AA metabolism, immune system activation can also be modeled using various stimulants. Commonly used compounds to simulate systemic inflammation may include bacterial derived lipopolysaccharide (**LPS**), complete Freund's adjuvant (**CFA**) to simulate local lung inflammation, and diquat or hydrogen peroxide to model and induce oxidative stress. Repeated injection with LPS was the most common method of immune stimulation (72% of immune stimulant studies, 31% of all

studies) and encompassed a wide BW range (2 to 65 kg BW). Compared to non-challenged controls, few studies reported decreases in growth performance and feed efficiency (van Heugten et al., 1994; de Ridder et al., 2012; Campos et al., 2014a; Campos et al., 2014b), N retention (Rakhshandeh et al., 2010; Campos et al., 2014b; Rakhshandeh, 2015; Rudar et al., 2016) and protein deposition (Campos et al., 2014b; Rudar et al., 2016; Rudar et al., 2017). Interestingly, N and AA digestibility were generally unaffected by LPS challenge compared to controls (Rakhshandeh et al., 2010; Litvak et al., 2013b; Campos et al., 2014b; Rakhshandeh et al., 2014). Evaluating changes in AA requirements in response to LPS has yielded mixed results. de Ridder et al. (2012) reported that increasing dietary Trp improved protein deposition in 20 kg BW pigs. Similarly, Rakhshandeh et al. (2010), Kim et al. (2012), and Litvak et al. (2013b) reported increases in N balance and protein deposition with increasing SAA in 22, 53, and 17 kg BW pigs, respectively, while Rakhshandeh et al. (2014) reported a decreased SAA requirement for protein deposition in 19 to 23 kg BW pigs.

Interestingly, inducing lung inflammation with intravenous CFA did not elicit changes in performance in any study (Melchior et al., 2004; Melchior et al., 2005; Le Floc'h et al., 2008; Kampman-van de Hoek et al., 2015) and decreased N retention in only one study (Kampman-van de Hoek et al., 2015); however, increased APP indicated immune stimulation (Kampman-van de Hoek et al., 2015). Using the intravenous CFA challenge model in pigs, much of the published research has primarily been focused around Trp metabolism, and although plasma Trp decreases no changes in requirements have been indicated (Melchior et al., 2004; Le Floc'h et al., 2008). Induction of oxidative stress with hydrogen peroxide or diquat has resulted in reduced pig performance (Lv et al., 2012; Zheng et al., 2013; Duan et al., 2016), and supplementing the diet with additional Glu, Asp, or Arg improves performance (Zheng et al., 2013; Duan et al., 2016).

This may suggest increased Glu, Asp, and/or Arg requirements for growth in an oxidative stress model.

As summarized in Table 1.4, a bulk of published research has involved supplementing dietary AA over NRC (2012) requirements with a focus on Arg, Trp, Thr, and SAA. Increasing these AA in the diet above requirement has proved beneficial for growth and feed efficiency (Capozzalo et al., 2012; Chen et al., 2012; Zheng et al., 2013; Ren et al., 2014; Capozzalo et al., 2015; Capozzalo et al., 2017; Jayaraman et al., 2017b), protein accretion (de Ridder et al., 2012; Kim et al., 2012; Litvak et al., 2013b), and N balance (Rakhshandeh et al., 2010; Kahindi, 2014; Mao et al., 2014; Capozzalo et al., 2017). Arginine, Trp, Thr, and SAA are of interest for their involvement in the immune response. Arginine and its metabolites are critical for the urea cycle, which deals with increased urea from the liver during disease, and Arg is used for nitric oxide synthesis, a potent antioxidant and vasodilator (Wu and Morris, 1998). Tryptophan serves as a precursor to serotonin which can regulate the stress response by reducing glucocorticoids and can mediate feed intake by increasing ghrelin concentrations (Le Floc'h and Seve, 2007). Threonine is one of the most limiting AA in practical swine diets and is important for the maintenance of intestinal structure, mucin and IgA synthesis, and protein synthesis (Ruth and Field, 2013). Sulfur amino acids (Met, Cys, Ser) and their metabolites are crucial for oxidative status through glutathione and can improve T-cell activity and reduce inflammation (Grimble, 2006).

Protein and Amino Acid Post-Absorptive Metabolism and Metabolic Adaptation to Stress and Disease

The activation of immune cells from a quiescent (resting) state, and subsequently, a reduction in feed intake may lead to alterations in AA metabolism. Also, increased synthesis of APP and cytokines occurs, and their AA composition differs from dietary or skeletal muscle composition (Reeds et al., 1994). The primary consideration of immune system metabolism, or "immunometabolism," is the role of metabolic pathways within immune cells and how these pathways regulate immune responses (Pearce and Pearce, 2013). The production of ATP is essential in both quiescent and activated immune cells. Glucose is the primary fuel used for ATP production through two pathways: glycolysis or oxidative phosphorylation. Glycolysis involves the conversion of glucose to pyruvate to generate ATP. Oxidative phosphorylation (**OXPHOS**) involves the tricarboxylic acid (**TCA**) cycle generating reducing equivalents to donate electrons to the electron transport chain to generate ATP. The TCA cycle can be used during glycolysis when pyruvate is converted to acetyl-CoA, which enters the TCA cycle. Immune cells can also use other fuel sources, like AA and fatty acids, to generate ATP. Glucogenic AA are generally converted to pyruvate or Gln (Fig. 1.4) which, through

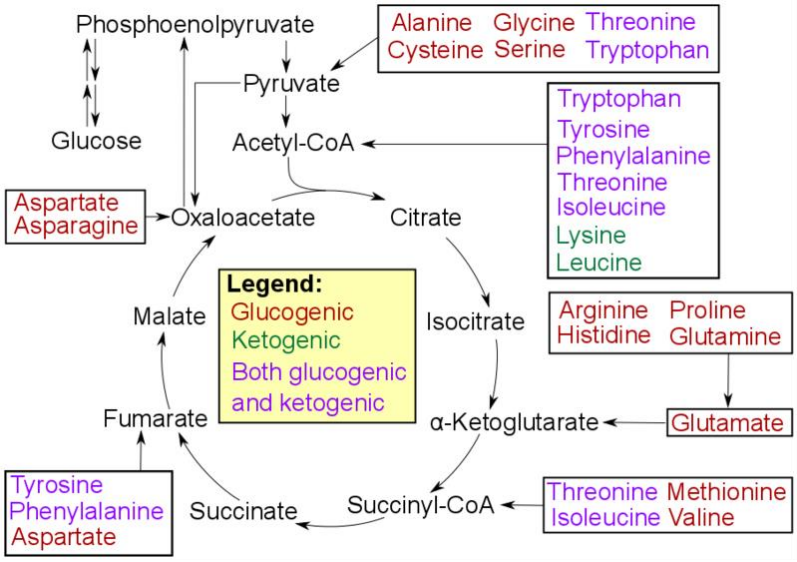

Figure 1.4. Glucogenic and ketogenic amino acids.

glutaminolysis, fuels OXPHOS. Some immune cell types utilize aerobic glycolysis (glycolysis even in the presence of oxygen) for ATP generation in quiescence, and many cell types metabolically convert from OXPHOS to aerobic glycolysis upon activation (Everts et al., 2012; Pearce and Pearce, 2013; O'Neill, 2014). Interestingly, memory immune cells rely more on fatty acid oxidation and OXPHOS than aerobic glycolysis.

Reduced feed intake appears necessary for survival during disease (Murray and Murray, 1979; Tsat et al., 1994). Shortly after a pathogen is detected, proinflammatory cytokines are released and act on the hypothalamus, reducing feed intake and facilitating protein degradation from lean tissue (Johnson, 1997, 1998). Decreased feed intake leads to a reduction in the amount of nutrients available for the animal which is compounded by decreases in digestibility and availability often associated with disease. Therefore, body stores of nutrients are catabolized to provide AA and energy substrates to maintain cellular processes and for protein synthesis. This occurs through energy-generating mechanisms, namely gluconeogenesis, ureagenesis, and ketogenesis. As explained previously, AA can be act as precursors for glucose, and gluconeogenesis, or ketone bodies. Ketones are generated from the liver during fatty acid degradation and can be used as an energy source for peripheral tissues. It appears that pigs can become ketotic when experiencing stress and reduced feed intake (Perri et al., 2016), as with weaning, although the extent to which immune cells and peripheral tissues utilize ketone bodies for energy is unknown.

When in a negative energy or N balance, pigs catabolize lean tissue to generate substrates for gluconeogenesis. Large amounts of amino-N are generated in response to infection for use by the liver as a consequence of metabolic response to infection which includes: increased muscle proteolysis, increased Ala production from branched-chain AA (**BCAA**) in muscle, increased

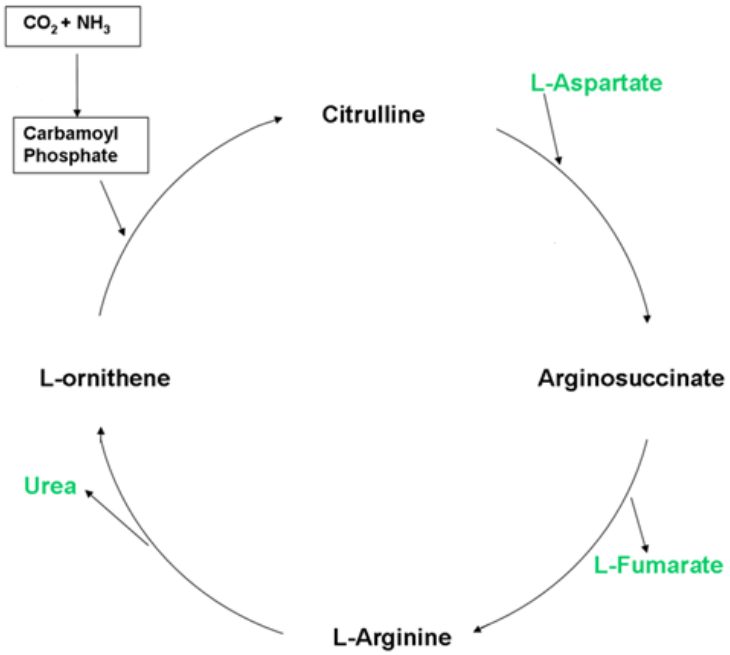

Figure 1.5. Urea cycle

Ala release from muscle, increased plasma AA uptake in the liver, and increased glucose derived from AA in the liver (Beisel and Wannemacher, 1980). This culminates in an increase in urea production, which is toxic at elevated levels, and therefore, must be excreted through the urea cycle (Fig 1.5) or synthesized into glutamine (Dimski, 1994). The urea cycle occurs in the liver, where ammonia and bicarbonate form carbamoyl phosphate which reacts with ornithine to form citrulline. Citrulline and Asp react, forming arginosuccinate which is split into fumarate, a TCA cycle intermediate, and Arg. Arginine is then split into ornithine and urea, which is excreted in the urine. Urea synthesis is an energy requiring process, therefore, it is more energetically efficient to use lactate as a substrate (Beisel and Wannemacher, 1980). A second major pathway of ammonia metabolism is glutamine synthesis. This is a high-affinity system compared to the low-affinity, high capacity urea cycle and acts as a backup system for ammonia detoxification, in which ammonia reacts with glutamate to form glutamine and is catalyzed by glutamine synthetase.

These changes in metabolism during times of disease or negative energy balance demonstrate areas that can be exploited to provide adequate substrate through the diet and lead to increased nutrient availability for immune function or protein synthesis. Increasing glucogenic AA and AA involved in the urea cycle could improve growth or feed efficiency during disease.

Conclusions

In the data summarized above, growth and protein accretion are clearly impacted by disease, suggesting changes in AA pre- and post-absorptive metabolism during periods of immune and health challenge. It is also demonstrated that supplementing the diet with different AA above requirements and altering energy utilization can improve disease outcomes. Several studies have focused on understanding the impact of bacterial enteric infection on the small intestine, primarily pathogenic *E. coli*, and AA requirements that surround its pathogenesis and resolution. There are also several studies that have studied metabolic AA changes when immune stimulation is modeled. Collectively, Table 1.4 highlighted that requirements for Lys, Arg, Trp, and SAA may be changing, indicating the order of limiting AA in immune-stimulated or health-challenged pigs may differ from healthy pigs. However, a better understanding of AA nutrition and metabolism under stress is needed so producers can better formulate diets to optimize disease resolution, pig well-being, and performance, ultimately recovering costs lost to disease.

The NRC reports AA requirement recommendations on a SID basis, and outside of two studies, SID values have not been established for pigs infected with pathogens affecting the swine industry. This is an area of opportunity to better define and understand AA metabolism and nutrition during times of disease, specifically during live viral challenges. There is no available data quantifying BAAL for pathogens other than *Salmonella* Typhimurium. Therefore, to better understand AA nutrition during disease, this dissertation will focus on:

- Alterations in AID and characterization of SID values in response to PRRSV or Bhyo challenge
- Endogenous AA losses associated with PRRSV or Bhyo challenge
- Changes in Lys:ME requirement during PRRSV infection in grower pigs

Table 1.4. Changes in amino acid or crude protein metabolism under different models of health challenges

Challenge	BW	Performance	AA metabolic changes	Study
Systemic inflammation				
LPS	21 kg	NR	↓ serum total protein ↓ plasma Gly, Gln, Tyr, total BCAA ↑ plasma Phe	Bruins et al. (2002)
LPS	55 kg	↓ ADG, ADFI, G:F	↓ glutathione, T_3, T_4	Campos et al. (2014a)
LPS + HS	55 kg	↑ G:F; ↓ ADG, ADFI	↓ glutathione, T_4	Campos et al. (2014a)
LPS	65 kg	↓ ADG, ADFI	↔ N ATTD ↓ N retention; ↓ protein deposition	Campos et al. (2014b)
LPS	20 kg	↓ ADG; ↔ ADFI	↑ Trp for protein deposition	de Ridder et al. (2012)
LPS	14 kg	↔ ADG, ADFI	↓ SI architecture; ↑ mucosal ornithine	Hou et al. (2010)
LPS	53 kg	NR	↑ SAA:Lys for protein deposition (at least 138% of control requirement)	Kim et al. (2012)
LPS	10	NR	↔ APP; FSR liver, loin, intestine, spleen ↑ plasma FSR	Litvak et al. (2013a)
LPS	17	↔ ADG	↔ ATTD N ↑ APP, ↓ serum albumin ↑ Met:Met+Cys for protein deposition (ISS1 = 109%; ISS2 = 104%)	Litvak et al. (2013b)
LPS	2 kg	NR	↓ plasma BCAA ↓ FSR glycolytic muscle tissue (LD, gastrocnemius) ↔ FSR oxidative muscle tissue, GIT, lung ↑ FSR liver, spleen, kidney, diaphragm (mixed glycolytic/oxidative)	Orellana et al. (2004)
LPS	9 kg	↔ N intake	↓ AID N, N balance ↓ ILR Lys, Phe; ↑ BUN	Rakhshandeh (2015)

Table 1.4 continued

Challenge	BW	Performance	AA metabolic changes	Study
LPS	22 kg	↔ ADG	↑ APP	Rakhshandeh and de Lange (2012)
LPS	22 kg	↔ N intake	↔ AID N, AA; ↓ N retention, N/S balance ↑ SAA for N balance	Rakhshandeh et al. (2010)
LPS	19 or 23 kg	↔ ADFI	↔ AID GE; SID N, Lys, Met, Met+Cys, Thr, Arg, Ile, Leu ↓ SAA for protein deposition (8%)	Rakhshandeh et al. (2014)
LPS	14 kg	↔ N intake	↓ N retention, protein deposition, PUN	Rudar et al. (2016)
LPS	11 kg	↔ N intake	↓ whole-body protein synthesis, protein deposition ↑ protein synthesis:deposition	Rudar et al. (2017)
LPS	6 kg	↓ ADG, G;F	↓ gain:protein intake	van Heugten et al. (1994)
LPS	7 kg	NR	↑ aspartate aminotransferase activity; plasma Lys, Ala ↓ hepatic GPx activity	Wang et al. (2015)
Lung inflammation				
Complete Freund's adjuvant	38 kg	NR	↑ APP, serum total protein ↔ ATTD N, ↓ N retention ↓ ILR Val, Tyr	Kampman-van de Hoek et al. (2015)
Complete Freund's adjuvant	12 kg	↔ ADG	↓ plasma Trp	Le Floc'h et al. (2008)
Complete Freund's adjuvant	14 kg	↔ ADG	↔ plasma Trp, kynurenine	Melchior et al. (2005)
Complete Freund's adjuvant	11 kg	↔ ADG; ↓ ADFI	↓ plasma Trp, Gln, Pro, Gly, Tyr, total AA ↑ plasma His	Melchior et al. (2004)
Oxidative Stress				
Hydrogen peroxide	11 kg	↓ ADG, FCR	↑ serum SOD, SI AA transporters ↑ portal Ile, Phe, Val, His; serum Val ↓ portal Ser, Met, Pro, Glu, Gly, Ala, Lys; serum Thr, Ser, Met, Tyr, Pro ↑ Glu, Asp for ADG	Duan et al. (2016)

Table 1.4 continued

Challenge	BW	Performance	AA metabolic changes	Study
Diquat	11 kg	↓ ADG, ADFI, G:F	↓ catalase, SOD, GPx activity ↑ γ-glutamyl transpeptidase ↓ serum Trp; ↑ serum kynurenine ↔ serum LNAA, SI AA transporter	Lv et al. (2012)
Diquat	9 kg	↓ ADG, ADFI	↔ plasma Arg ↓ SI architecture; AA transporters ↑ Arg for ADG	Zheng et al. (2013); Zheng et al. (2017)
Unclean/unsanitary environment				
Unsanitary environment	7 kg	↓ ADG, ADFI	↓ plasma Thr; ↑ plasma Lys, PUN ↔ Thr:Lys requirement	Jayaraman et al. (2015)
Unsanitary environment	7 kg	↓ ADG, G:F ↔ ADFI	↔ Trp:Lys requirement; PUN ↓ SI architecture; ↔ V:C	Jayaraman et al. (2017a)
Unsanitary environment	25 kg	↔ ADG, ↑ G:F	↓ ATTD DM, N ↑ N retention ↑ ILR Lys	Kampman-van de Hoek et al. (2016)
Unsanitary environment	17-110 kg	↓ ADG, G;F ↔ ADFI	↓ ATTD N; ↑ pleuritis score, pleuritis lung %	van der Meer et al. (2016)
Unsanitary environment	8 kg	↓ ADG, ADFI, G:F	↓ plasma Trp ↔ plasma glutathione	Le Floc'h et al. (2009)
Unsanitary environment	8 kg	↓ ADG, ADFI, G:F	↔ plasma Trp ↔ Trp:Lys for ADG (0.205)	Le Floc'h et al. (2010)
Unsanitary environment	6-27 kg	↓ ADG, G:F ↔ ADFI	↓ protein accretion ↑ Lys for ADG, protein accretion	Williams et al. (1997b)
Unsanitary environment	6-27 kg	↓ ADG, ADFI ↔ G:F	↓ ATTD N, N retention, Lys utilization ↓ Lys for ADG, G:F, N retention	Williams et al. (1997a)
Unsanitary environment	6-112 kg	↓ ADG, ADFI, G:F	↓ muscle, protein accretion ↓ Lys for ADG, G:F	Williams et al. (1997c)
Unsanitary environment	7 kg	↓ ADG, ADFI; ↔ G:F	↑ Lys for ADG, G:F	Kahindi et al. (2013)

Table 1.4 continued

Challenge	BW	Performance	AA metabolic changes	Study
Unsanitary environment	7 kg	↔ADG, ADFI, G:F	↔ SAA:Lys for G:F ↑ SAA:Lys for PUN	Kahindi (2014)
Live pathogen				
ETEC	6 kg	↔ ADG, ADFI, G:F	↔ plasma Trp, kynurenine ↑ Trp:Lys for G:F	Capozzalo et al. (2012)
ETEC	6 kg	NR	↑ plasma Trp, Val, Pro, Arg, Ile, Thr, Phe, Ser, Ala, Asp, Tyr ↓ plasma Lys, Leu, His, Met ↑ Trp:Lys for G:F; SAA:Lys for G:F, N balance	Capozzalo et al. (2017)
ETEC	8 or 13 kg	↔ ADG, ADFI, G:F	↔ SI architecture	Wellock et al. (2008a, 2008b)
ETEC	6 kg	↔ ADG, ADFI, FCR	↔ plasma Trp, urea ↑ Trp:Lys for FCR	Capozzalo et al. (2015)
ETEC	7 kg	↔ ADG, ADFI, G:F	↔ SAA:Lys (minimum 0.54)	Kahindi (2014)
ETEC	5 kg	↔ ADG, ADFI, G:F	↔ CP for performance ↓ CP for SI health	Opapeju et al. (2009)
ETEC	7 kg	NR	↔ PUN ↓ CP reduced diarrhea, intestinal ammonia	(Heo et al., 2010a)
ETEC	7 kg	↓ ADG, ↑ G:F ↔ ADFI	↓ AID N, all AA ↓ SI architecture ↔ PUN ↓ CP reduced diarrhea, intestinal ammonia	(Heo et al., 2010b)
E. coli K88	6 kg	↔ ADG, ADFI, G:F	↑ Trp:Lys for ADG (21.7%), G:F (20.1%)	Jayaraman et al. (2017b)
E. coli K88	7 kg	↓ ADG, FCR	↓ SI architecture ↑ SID Thr for ADG, FCR, SI architecture	Ren et al. (2014)
E. coli K88ac	8 kg	↔ ADG, ADFI	↔ SI architecture	Trevisi et al. (2015)
E. coli K88ac	7 kg	↔ ADG, ADFI	↔ SI architecture	Trevisi et al. (2009)

Table 1.4 continued

Challenge	BW	Performance	AA metabolic changes	Study
E. coli K88	2 kg	↓ ADG, ↔ ADFI	↔ plasma AA ↑ plasma IgA, IgG, IgM ↑ SI secreted IgA	Zhang et al. (2013)
Salmonella Typhimurium	18 kg	↓ ADG, G:F; ↔ ADFI	24-h: ↓ AID Ile, Gly; ↑ SID His, Gly ↑ BAAL all EAA, NEAA 72-h: ↓ AID Lys, Phe, Thr, Ser ↔ BAAL; ↓ SID Lys	Lee (2012)
Salmonella Typhimurium	76 kg	NR	8-16 h: ↓ AID; ↑ BAAL; ↓ SID 56-64 h: ↔ BAAL, AID, SID 72-80 h: ↓ BAAL, AID, SID	
attenuated *Salmonella* Cholerasuis	7 kg	↓ ADG, ADFI ↔F:G	↑ CRP; ↓ serum Arg ↑ Arg for ADG, ADFI	Chen et al. (2012)
Brachyspira hyodysenteriae	23 kg	↓ ADG	↓ plasma Ala, Gln, Tyr, Ser, Asp, Tau ↑ plasma Lys, Ile	Jonasson et al. (2007)
Virus or vaccine				
PRRSV	9 kg	↓ N intake	↓ AID N, N balance; ↑ plasma creatinine; ↑ ILR Met, Thr	Rakhshandeh (2015)
PRRSV	16 kg	↓ ADG, ADFI; ↔ G:F	↔ AID, ATTD N; AID AA, BUN ↑ SI active Lys transport ↔ SI aminopeptidase activity	Schweer et al. (2016a); Schweer et al. (2016b)
PEDV	16 kg	↓ ADG, ADFI; ↔ G:F	↔ ATTD N; AID N, AA; ↑ BUN ↓ SI architecture ↑ SI active Gln transport ↔ SI aminopeptidase activity	
PRRSV + PEDV	16 kg	↓ ADG, ADFI, G:F	↓ ATTD N; ↔ AID N, AA ↑ BUN; ↓ SI architecture ↔ SI active Lys, Gln transport, aminopeptidase activity	
modified live PRRS vaccine	29 kg	↓ ADG, ADFI ↔ F:G	↑ serum EAA, NEAA except ↓ Phe and ↔ Tyr	Xu et al. (2014)

Table 1.4 continued

Challenge	BW	Performance	AA metabolic changes	Study
live Pseudorabies vaccine	7 kg	↔ ADG, ADFI, G:F	↑ serum Thr, Val, urea ↑ Thr for immune response, N balance	Mao et al. (2014)
attenuated Swine Fever vaccine	17.5 kg	NR	↑ Thr for immune response	Defa et al. (1999)

Increase (↑), decrease (↓), no change (↔), or not reported (NR) in parameter
APP = acute phase protein; CRP = C-reactive protein; LNAA = large neutral AA; ETEC = enterotoxigenic *E. coli*; GPx = glutathione peroxidase; SI = small intestine; SOD = superoxide dismutase
ILR = irreversible loss rate, increase = use for protein synthesis or oxidation
FSR = fractional synthesis rate, percent of protein mass synthesized in a day
Protein synthesis:deposition = amount of protein synthesized per protein accreted, indicates energetic efficiency of protein deposition
Lys utilization = g/d per g daily digestible Lys intake

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CHAPTER 2. THE IMPACT OF PRRSV INFECTION AND DIETARY SOYBEAN MEAL INCLUSION ON ILEAL AMINO ACID DIGESTIBILITY AND ENDOGENOUS AMINO ACID LOSSES IN GROWING PIGS

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is a significant disease in the swine industry, and increasing soybean meal (SBM) consumption during this disease challenge may improve performance. Our objectives were to determine the impact of SBM level on apparent total tract (ATTD) and ileal (AID) digestibility during PRRSV infection and to determine ileal basal endogenous losses (BEL) during PRRSV infection. Forty PRRSV negative gilts were fitted with a T-cannula in the distal ileum. Treatments were arranged in a 2 × 2 factorial with high and low SBM (HSBM, 29% vs. LSBM, 10%), with and without PRRSV (n=6/treatment). The remaining pigs (n=8/challenge status) were fed a N-free diet. Chromic

oxide was used as an indigestible marker. On day post inoculation (dpi) 0, at 47.7 ± 0.57 kg BW, 20 pigs were inoculated with live PRRSV; 20 control pigs were sham inoculated. Infection was confirmed by serum PCR. Feces were collected at dpi 5-6 and 16-17, and ileal digesta collected at dpi 7-8 and 18-19. Feed, feces, and digesta were analyzed for DM, N, and GE. Digesta and feed were analyzed for AA. Data were analyzed in a 2 × 2 + 2 factorial design to determine main effects of diet and PRRSV and their interaction. Data from N-free fed pigs were analyzed separately to determine BEL and hindgut disappearance due to PRRSV infection. All control pigs remained PRRSV negative. There were no interactions for AID of AA; however, HSBM reduced DM, GE, Lys, and Met AID and increased Arg and Gly AID during both collection periods ($P < 0.05$). At dpi 7-8 only, PRRSV reduced DM and GE AID, ($P < 0.05$). At 7-8 dpi, BEL of Arg, Ala, and Pro were reduced ($P < 0.05$) due to PRRSV by 64, 39, and 94%, respectively. At dpi 18-19 BEL of Thr tended ($P = 0.06$) to be increased in PRRSV infected pigs; however, no other differences were observed. Pigs fed LSBM had increased Lys, Met, Thr, Trp, and Pro SID, primarily at 7-8 dpi. At 7-8 dpi, PRRSV reduced Arg, Gly, and Pro SID ($P < 0.01$), and SID Pro continued to be reduced by 17% at dpi 18-19. Compared to HSBM pigs, LSBM reduced hindgut disappearance of DM and GE at dpi 5-8 and 16-19 while N disappearance was reduced at dpi 5-8. There were no differences between control and PRRSV N-free fed pigs. Altogether, SBM inclusion impacts SID of AA and hindgut disappearance of nutrients, regardless of PRRSV. In contrast, there is minimal impact of PRRSV on BEL, and therefore, SID of most AA are not different.

Keywords: amino acids, digestibility, endogenous losses, pig, PRRS

Introduction

Tissue accretion rates and performance efficiency of health challenged pigs are reduced (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017), suggesting an alteration in nutrient utilization and resource allocation (Rakhshandeh et al., 2010; Rauw, 2012). As such, attention has been given to nutritional intervention strategies to improve the health, well-being, and performance of pigs. Recently, one strategy has involved increasing dietary soybean meal (**SBM**), and thus reducing crystalline AA use, which has been touted to promote more rapid disease resolution and improve growth performance and feed efficiency during viral pathogen challenges (Boyd et al., 2010; Rochell et al., 2015). However, the mode of action by which these beneficial SBM effects may occur are poorly defined and may involve nutrient digestibility (Schweer et al., 2017) or bioactive compounds associated with SBM (Greiner et al., 2001a, b).

Porcine reproductive and respiratory syndrome virus (**PRRSV**) is one of the most economically significant swine diseases in the world, costing the US pork industry more than $660 million annually (Holtkamp et al., 2013). In growing pigs, PRRSV reduces growth performance and feed efficiency (Escobar et al., 2004; Schweer et al., 2016b). Reduced apparent total tract digestibility (**ATTD**) of nutrients and energy in grow-finisher pigs challenged with PRRSV has also been reported (Schweer et al., 2017); however, in nursery pigs it has been shown that PRRSV did not alter ATTD or apparent ileal digestibility (**AID**) of nutrients, energy, or AA after experimental infection (Schweer et al., 2016b).

Interestingly, basal endogenous AA losses (**BEL**) have not been quantified in relation to a PRRSV challenge, and thus it is not known if standardized ileal digestibility (**SID**) of N or AA would be different. Even so, limited studies have determined the BEL of AA due to a pathogen or vaccine challenge in pigs or other livestock species. In nursery and growing pigs, *Salmonella*

Typhimurium increased BEL of several AA (Lee, 2012). In contrast, use of a mild coccidial vaccine in broilers reduced BEL of several AA (Adedokun et al., 2012). Therefore, the objectives of this study were to determine how PRRSV infection impacts the digestibility of nutrients and energy in high and low SBM diets and to determine BEL of AA in response to PRRSV infection in growing pigs.

Materials and Methods

Animals, Housing and Experimental Design

All animal work was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 1-16-8156-S) and adhered to the ethical and humane use of animals for research.

The experiment was performed in two identical replicates consisting of 20 gilts each. In total, 40 gilts (38.6 ± 0.70 kg BW) negative for PRRSV as determined by PRRS PCR and X3 ELISA (Iowa State University Veterinary Diagnostic Laboratory, Ames, IA), were selected and surgically fitted with a T-cannula in the distal ileum as previously described (Stein et al., 1998). After surgery, pigs were moved to individual pens (1.8 × 1.9 m) and allowed to recover for 10-14 d. Following the recovery period, pigs were semi-sedated with 1.1 mg/kg BW of a tiletamine-zolazepam-ketamine-xylazine (Fort Dodge Animal Health, Fort Dodge, IA) combination for safe transport to the BSL2 Livestock Infectious Disease Isolation Facility (**LIDIF**) at the Iowa State Veterinary College (Ames, IA). Pigs were individually penned (1.4 × 1.5 m) with each disease status having a separate room (Control or PRRSV) to prevent viral cross-infection. Following a 4 d adaptation period at the LIDIF, on day post inoculation (**dpi**) 0, pigs in the PRRSV room (n=10 pigs/rep) were inoculated with 2 mL (1 mL i.m. and 1 mL intranasal; 10^6 genomic units per mL)

of a live PRRSV (open reading frame 5 sequence 1-3-4), while the Control room (n=10 pigs/rep) received a sham saline inoculation. At the start of the first and second collection period, grower pigs with a BW of 47.7 ± 0.57 and 50.2 ± 0.99 kg, respectively, were used.

Diets and Feeding

Dietary treatments were arranged in a 2 × 2 + 2 factorial with two SBM dietary inclusion (10% versus 29.7%) by PRRSV challenge status (with or without) as factorial variables plus a N-free (**NF**) diet with or without PRRSV as an added variable. Dietary treatments included a high SBM (**HSBM,** 29.7% SBM; n=6 pigs/challenge status) and low SBM (**LSBM,** 10.0% SBM; n=6 pigs/challenge status) diet (Table 2.1) that met or exceeded requirements for nutrients and energy (NRC, 2012). The 29.72% SBM was chosen and considered high because this inclusion rate met all the essential AA requirements without the addition of crystalline AA for this size pig. Further, going beyond this inclusion rate of SBM would promote excess N excretion and wastage. Soybean meal inclusion was limited to 10% in the LSBM diet and supplemented with L-Lys·HCl. Diets were formulated to be isocaloric (ME basis) and contain similar SID Lys concentrations (Table 2.1). At inoculation, a subset of pigs (n=8 pigs/challenge status) were allotted to an NF diet (Table 2.1) to determine BEL associated with PRRSV. All diets contained 0.40% chromic oxide as an indigestible marker. Pigs were allotted to diets based on BW and diets were fed starting post-surgery. Pigs on the HSBM and LSBM diets were fed the same diet for the duration of the experiment. The NF diet was fed at 0-8 dpi and 12-19 dpi (4-5 d diet adaptation followed by 4 d collection). A 50-50 blend of HSBM and LSBM diets (Table 2.1) was fed after collection on 8 dpi through 11 dpi.

Pigs were restrictively fed to ensure the entire meal was eaten during the collection periods. Pigs were weighed before each collection period and the amount of feed provided at

each meal was recorded. For 5 d before collections, pigs were fed 2.5 times the estimated energy requirement for maintenance (2.5 × 197 kcal of ME per $BW^{0.60}$ (NRC, 2012)). The daily feed allotment was provided in 2 equal meals at 0700 and 1700 h.

Blood Collection and Analysis

To confirm PRRS viremia or the absence thereof, blood samples (10 mL) were collected from all pigs at dpi 0, 7, 14, and 21 via jugular venipuncture in vacutainer serum tubes (BD, Franklin Lakes, NJ) while pigs were restrained by a snare. After clotting, serum was separated by centrifugation (2,000 × *g*, 15 min at 4° C), aliquoted, and submitted to the Iowa State Veterinary Diagnostic Laboratory (Ames, IA) for PRRSV real-time RT-PCR and serology analysis. Testing for PRRSV was performed using commercial reagents (VetMAX™ NA and EU PRRSV real-time RT-PCR, Thermo Fisher Scientific, Waltham, MA). A commercial ELISA kit (HerdCheck® PRRS X3, IDEXX Laboratories, Inc., Westbrook, ME) was used to detect anti-PRRSV antibody per manufacturer's instruction.

Digesta And Fecal Sample Collection, Analysis, And Calculations

A representative feed sample from each diet was obtained from both replicates and pooled for subsequent analysis. In order to determine how peak viremia and seroconversion of PRRSV altered digestibility, digesta and feces were collected at two different periods. Feces were collected from all pigs on dpi 5-6 and 16-17 and pooled by pig within period. Ileal digesta was collected from 0800 to 1600 h on dpi 7-8 and 18-19 by attaching a 207-mL plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed when they were filled with digesta or every 30 min, whichever occurred first. All fecal samples were stored at -20°C until further analysis. Digesta samples were stored on dry ice at the BSL2 facility during collections and transferred to -20°C after each collection day.

At the end of each sampling period, ileal and fecal samples were thawed and mixed thoroughly within pig and sampling period. A subsample of ileal digesta was collected, stored at -20°C and lyophilized (Model 10-100; Virtis Co. Ltd., Gardiner, NY) to a constant weight. Fecal samples were dried in a mechanical convection oven at 100°C. Feed, fecal, and digesta samples were ground through a 1 mm screen (Model ZM1; Retsch Inc., Newton, PA) prior to analysis. Proximate analysis of feed, feces, and ileal digesta samples were analyzed as previously described (Stein et al., 2007; Oresanya et al., 2008). Briefly, all samples were analyzed for DM (method 930.15; (AOAC, 2007)), chromic oxide as described by Fenton and Fenton (1979), N using TruMac N (Leco Corporation, St. Joseph, MO), and GE using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Amino acid composition of diet and digesta samples was determined by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri-Columbia (Columbia, MO) by cation-exchange HPLC (L8900 Amino Acid Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

The AID (%) and ATTD (%) of each dietary component were calculated using the following equations (Oresanya et al., 2008):

AID or ATTD = 100 – [100 × (concentration of Cr_2O_3 in diet × concentration of component in feces or digesta ÷ concentration of Cr_2O_3 in feces or digesta × concentration of component in diet)]

The BEL of AA and N (g/kg DMI) were calculated using the following equation (Stein et al., 2007):

BEL = [AA or N in digesta × (Cr_2O_3 in diet ÷ Cr_2O_3 in digesta)]

Standardized ileal digestibility values for each AA were calculated by correcting the AID for BEL using the equation (Stein et al., 2007):

SID = [AID + (BEL ÷ AA in diet)]

As this was not a crossover design each pig could not serve as its own control for SID determination; therefore, statistical analysis was performed on the BEL values and the reported treatment averages were used to determine SID values.

Disappearance of DM (g/d), N (g/d), and GE (Mcal/d) in the hindgut was calculated using the following equation (Pilcher et al., 2013):

Hindgut disappearance = amount remaining at terminal ileum – amount excreted in feces

Statistical Analysis

The 40 pigs were assigned to a 2 × 2 + 2 factorial design. Start BW were equal among treatments, and the data were analyzed as a completely randomized design using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc. Cary, NC). A 2 × 2 factorial design was used to compare the fixed effects of SBM inclusion (10% versus 29.7% dietary SBM), PRRSV (challenge versus non-challenge), and their interaction on AID, SID, and hindgut disappearance of nutrients and energy near peak PRRS viremia (dpi 5-8) and seroconversion (dpi 16-19). Control and PRRSV pigs fed NF diets were analyzed separately from the factorial design using the same completely randomized design to determine the impact of PRRSV on BEL and hindgut disappearance of nutrients and energy. Pig was considered the experimental unit for all analyses. Replicate was used as a random effect. All data are reported as least squares means ± SEM and considered significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

Results and Discussion

Previous research completed by our group (Schweer et al., 2016b; Schweer et al., 2017) and others (Greiner et al., 2000; Escobar et al., 2004) have reported reduced growth performance and feed efficiency due to PRRSV infection. Additionally, protein and fat accretion are reduced during a PRRSV challenge both acutely (Escobar et al., 2004) and throughout the entire finishing period (Schweer et al., 2017). Dietary strategies are of interest to recover lost growth performance and promote earlier clearance of virus in pathogen challenged pigs. One such strategy has been the use of increasing dietary SBM. It has been reported in a commercial production environment, that increasing dietary SBM to 32% inclusion can improved growth performance during a natural inflammatory-pathogen challenge in the finishing period of pigs (Boyd et al., 2010). Similarly, in an experimental setting, increasing dietary SBM from 17 to 29%, reduced serum viremia load and improved growth in nursery pigs (Rochell et al., 2015). However, it remains unclear if improved performance and viral clearance is a result of increased digestibility of CP and AA, or by increasing the bioactive antioxidant compounds (i.e., isoflavones) that are found within SBM. Therefore, the objectives of the experiment presented herein were aimed to determine if increasing SBM level improved ileal digestibility of AA and to quantify BEL of AA during a PRRSV challenge. This allowed for AA SID coefficient calculation and then compared the SID AA values between healthy (NRC, 2012) and PRRSV challenged pigs.

In the first replicate of the experiment, one pig in the control HSBM treatment was removed from the study after the first collection period due to a cannula malfunction. In the second replicate, three pigs in the PRRSV NF treatment were removed from the study. Two of these pigs were euthanized due to severe interstitial pneumonia secondary to acute PRRSV

infection as determined by a veterinary diagnostician, and the other pig was removed due to excessive BW loss as defined in the IACUC. Data from these removed pigs were not used in the analysis. The calculated and analyzed nutrient concentrations in each diet are presented in Table 2.2. As expected due to diet formulation, the HSBM diet had increased CP (18.46 vs. 13.04%) compared to the LSBM diet; however, analyzed total dietary Lys was similar in both diets (1.12 vs. 1.10%, respectively) due to the use of crystalline AA.

Viremia and Antibody

All pigs were negative for PRRS virus and antibody prior to inoculation as determined by serum PCR and ELISA. As desired, control pigs remained PRRSV negative throughout the 21 d experimental period, and all PRRSV inoculated pigs had detectable levels of PRRS virus and antibody at 7, 14, and 21 dpi (Table 2.3). Expectedly, viremia decreased, and antibody increased from 7 to 21 dpi, respectively ($P < 0.001$), indicating pigs were clearing the virus and seroconverting antibodies. In the current study all PRRSV infected pigs, including PRRSV inoculated NF pigs, demonstrated a classical PRRS viremia and antibody (seroconversion) response based on the timing of viremia (by 7-14 dpi) and seroconversion (dpi 14-21). This is similar to what has been previously reported in growing pigs infected with PRRSV (Greiner et al., 2000; Zimmerman et al., 2012; Schweer et al., 2016b). Interestingly, there was no effect of dietary SBM inclusion ($P > 0.10$) on serum PRRS viremia or antibody response. This is in contrast to Rochell et al. (2015), who report HSBM diets decreased serum PRRS viral load at 14 dpi as determined by PCR Ct values; although these were younger pigs, the inclusion of SBM was similar to the current study, 29.0 vs. 29.7%, respectively.

Apparent Total Tract and Ileal Digestibility

To understand how viremia and seroconversion may alter digestibility of energy and nutrients, collection periods were chosen at 5-8 dpi and 16-19 dpi to coincide with peak PRRS viremia and seroconversion, respectively. Apparent total tract digestibility of DM, N, and GE was assessed from dpi 5-6 (Table 2.4) and dpi 16-17 (Table 2.5) by fecal grab sample. There was no diet × PRRSV interaction ($P > 0.10$) at either time point or any effect of PRRSV on any ATTD coefficients evaluated. No effect of PRRSV on ATTD coefficients is in agreement with a previous study from our group (Schweer et al., 2016b); however, this is in contrast with another study from our group (Schweer et al., 2017). In the later study, pigs were housed in a commercial barn and not in a BSL2 facility and could have been exposed to secondary pathogens. Together, this would have had a higher immunological burden that would have contributed to the reduction in ATTD coefficients reported in the field study. Expectedly, there was an effect of diet at both time points post-inoculation where ATTD of N was reduced in LSBM compared to HSBM ($P < 0.01$). This is in agreement with previous reports demonstrating that as dietary CP decreases, so does N digestibility (Yu et al., 2017). Also, at 5-6 dpi, ATTD of GE was reduced in the LSBM diet compared to HSBM ($P < 0.01$). This is in contrast to previous studies that reported no difference between high protein and low protein, AA-supplemented diets on energy digestibility (Kerr and Easter, 1995); however, these pigs were younger and not housed in BSL2 facilities.

Apparent ileal digestibility of DM, N, and GE was also assessed from dpi 7-8 (Table 2.4) to dpi 18-19 (Table 2.5). No diet × PRRSV interactions were found at either collection period ($P > 0.10$). During both collection periods, LSBM diets resulted in an increased DM AID compared to HSBM diets ($P < 0.05$). Similarly, at 7-8 dpi AID of GE was increased in LSBM fed pigs compared to HSBM ($P = 0.027$). There was an effect of PRRSV at 7-8 dpi for AID of DM and

GE ($P < 0.04$). Dry matter AID was reduced by PRRSV in the HSBM fed pigs by 8.4% and LSBM pigs by 3.1% while GE AID was reduced by 7.7% and 3.4% in the HSBM and LSBM pigs, respectively. At 18-19 dpi, DM and GE AID were not reduced due to PRRSV, which our group has previously reported in nursery age pigs (Schweer et al., 2016b).

Apparent ileal digestibility of AA was determined from dpi 7-8 and dpi 18-19 (Table 2.4 and 5, respectively). At 7-8 dpi, AID of Arg was minimally reduced in HSBM pigs infected with PRRSV (85.91 vs. 84.14%) and increased in LSBM pigs infected with PRRSV (80.81 vs. 83.07%, respectively) leading to a tendency for a diet × PRRSV interaction ($P = 0.063$). This trend, however, did not continue at 18-19 dpi. Similarly, during the first collection period there was a tendency ($P = 0.099$) for PRRSV to reduce AID of Thr; however, this trend was not seen at 18-19 dpi. The AID of Lys, Met, and Thr were increased at 7-8 dpi in LSBM pigs ($P < 0.03$). Interestingly, only the AID of Met was significantly increased ($P = 0.023$) in LSBM pigs at 18-19 dpi, while AID of Lys showed a strong tendency ($P = 0.052$) to be increased. There was a significant reduction ($P < 0.05$) in the AID of Arg and Gly and a tendency ($P < 0.10$) for Tyr to be reduced in LSBM fed pigs at both collection periods. There was also a reduction ($P < 0.05$) in AID of Asp and Pro at dpi 7-8, Ser at 18-19 dpi, and a tendency ($P = 0.081$) for reduction of Cys at 18-19 dpi due to the LSBM diet.

Irrespective of challenge, increased digestibility of Lys, Met, Thr, and Trp in the LSBM diet was expected, as the diet was supplemented with crystalline AA which are considered 100% digestible (Chung and Baker, 1992). Although there was a tendency for Thr AID to be reduced by PRRSV in the first collection period, differences in AID of AA were not expected based on a previous study where AID of AA were not different at 21 dpi of PRRSV challenge (Schweer et al., 2016b). The previous study, however, utilized younger pigs and a different, less virulent

PRRSV isolate. Similarly, when pigs were challenged with lipopolysaccharide to elicit immune system stimulation, no AID differences were reported (Rakhshandeh et al., 2010). After 24 h of *Salmonella* Typhimurium infection only AID of Gly was reduced, and at 72 h after infection AID of Lys, Phe, Thr, and Ser were reduced (Lee, 2012), suggesting that health challenge or immune stimulation has little impact on AID coefficients.

Basal Endogenous Losses

One of the primary objectives of this paper was to determine if a PRRSV challenge altered BEL of N and AA in grower pigs. Surprisingly, BEL of N and AA are very poorly understood and defined across health compromised livestock species. In a limited number of studies, endogenous secretions are altered due to the enteric challenges *Salmonella* Typhimurium (Lee, 2012) and *Brachyspira hyodysenteriae* (Wilberts et al., 2014; Quintana-Hayashi et al., 2015), but in general, they are not well characterized. Basal endogenous loss of AA and N in healthy control pigs and pigs infected with PRRSV at 7-8 and 18-19 dpi were determined using the N-free method (Table 2.6). At 7-8 dpi, significant reductions ($P \leq 0.05$) in BEL of Arg, Ala, and Pro were detected; with no other differences noted ($P > 0.10$). Interestingly, BEL of N tended ($P = 0.087$) to be reduced in PRRS pigs; however, total tract basal N losses were increased in PRRS pigs (3.44 vs. 2.50 g/kg DMI, $P < 0.001$). At 18-19 dpi there was a strong tendency ($P = 0.057$) for BEL of Thr to be increased. There were also numerical reductions in BEL of Arg, Ala, and Pro during this collection period, but because of high variability significance was not detected. This high variability could be a result of variance associated with host-pathogen interactions, pathogen virulence and clearance rates, or small sample size. Similarly, ileal and total tract basal N losses were not different at 18-19 dpi.

When using an NF diet, BEL of Pro and Gly are generally overestimated (de Lange et al., 1989; Moughan et al., 1992), and there is an increase in BEL of Pro when pigs are offered NF diets for extended periods (Jansman et al., 2002); however, at both collection periods BEL of Pro and Ala were reduced in PRRSV infected pigs. This could suggest that infected pigs require more Ala and Pro than non-infected pigs. Collagen is abundant in the lungs, forming the bronchovascular skeleton and is also found in the lining of basal membranes, and is rich in Ala, Pro, and hydroxyproline (Eyre and Muir, 1975). Girard et al. (2001) reported that PRRSV increases collagenase activity in the lung at 7 and 14 dpi, which could increase the need for Ala and Pro. Basal endogenous loss of Arg was also reduced due to PRRSV. Arginine can be readily converted to Glu, a preferred energy substrate of activated immune cells (Maciolek et al., 2014), or Pro which is involved in collagen synthesis, as previously mentioned. Nitric oxide (**NO**), a derivative of Arg, exhibits antiviral activity; however, there are contrasting reports on the ability of NO to inhibit PRRSV replication (Pampusch et al., 1998; Jung et al., 2010; Yan et al., 2017). This could be a result of insufficient Arg causing NO inhibition which leads to increased reactive oxygen species and ultimately apoptosis (Lee and Kleiboeker, 2007).

The tendency for reduced BEL of N in PRRSV challenged pigs could suggest a reduction in the secretion of endogenous proteins such as mucins or trefoil factors, although mucins were not different at 21 dpi in a previous study (Schweer et al., 2016a). Digestive enzyme secretion could also be reduced, and although this requires further exploration, we have seen no reduction in sucrase, maltase, or aminopeptidase activities in the jejunum of PRRSV infected nursery pigs (Schweer et al., 2016a). Differences in total tract endogenous N loss could likely be related to microbial density and activity in the cecum and colon. Total microbial diversity can be reduced while proteolytic species (e.g., Proteobacteria), can increase in pigs severely impacted by

PRRSV challenge (Niederwerder et al., 2016). Similarly, pigs with increased microbial diversity and density in the gut had reduced coughing, lung lesion scores, and respiratory cytokines during *Mycoplasma hyopneumoniae* challenge (Schachtschneider et al., 2013). Changes in pig gut microbial density or diversity have not been described in other viral respiratory challenges.

Standardized Amino Acid Digestibility

The SID of AA was determined by correcting the AID coefficients for BEL at 7-8 dpi and 18-19 dpi (Table 2.7 and 2.8, respectively). There was a tendency for interaction ($P = 0.061$) at 7-8 dpi for the SID of Pro, where it was lower in HSBM pigs compared to LSBM and reduced by PRRSV in a similar manner (43 and 46% reduction, respectively) in both diets. At 18-19 dpi, no interactions were detected. A reduction ($P < 0.05$) in the SID of Arg, Gly, Pro, Ala ($P = 0.09$), and Ser ($P = 0.06$) from PRRSV infection was detected at 7-8 dpi. At 18-19 dpi only, a reduction in the SID of Pro ($P = 0.001$) was reported. An increase ($P < 0.05$) in SID of Lys, Met, and Trp in LSBM pigs was detected at both time points, while SID of Thr was increased ($P < 0.01$) at 7-8 dpi and tended ($P = 0.10$) to be increased at 18-19 dpi. Also, at 7-8 dpi, SID of Pro was significantly increased ($P < 0.001$) while Leu ($P = 0.096$) and Glu ($P = 0.077$) tended to increase in pigs fed the LSBM diet.

Interestingly, very few studies have examined the relationship between AA SID and infection in livestock species. In the current study, SID values were determined from AID values through the use of an NF diet and determination of BEL. As previously mentioned, BEL of some AA can be overestimated using an NF diet. Therefore, it is possible that some SID values can be overestimated. Proline determination can be variable; even so, there was a tendency for interaction in the current study. Decreased SID of Arg and Pro due to PRRSV infection are likely due to the decreased BEL of each of these AA. In a repeated lipopolysaccharide injection model,

Rakhshandeh et al. (2014), reported no difference in SID of Met and Cys; however, SID values were calculated from BEL values described by Jansman et al. (2002). To the author's knowledge, there are only two studies that report both BEL and SID values in pigs utilizing a *Salmonella* Typhimurium challenge model in nursery and grower pigs. In nursery pigs, Lee (2012) used a comparative slaughter technique and reported a tendency for SID of Arg to be reduced at 24 and 72 h post challenge. In the same study, and in contrast to the current study, SID of Gly was increased by more than two-fold at 24 h but was not different at 72 h post challenge. No differences in Pro, Ala, or Ser were reported in the study. Using the T-cannula method, SID of all AA were significantly reduced or tended to be reduced between 8 and 24 h after inoculation in growing pigs; however, by 56 h post inoculation, SID values had recovered to pre-inoculation values (Lee, 2012). In the same study, the greatest reduction was seen in Gly (53% reduction) which is in agreement with the current study but contrasts the previous study by Lee which utilized younger pigs. As *Salmonella* Typhimurium is a bacterial pathogen that impacts the intestinal tract, it likely has a different impact than a respiratory virus like PRRSV, likely leading to differences in the two studies.

Expectedly, SID of Lys, Met, Thr, and Trp were increased in the LSBM diet due to the use of crystalline AA which are assumed to be 100% digestible (Chung and Baker, 1992). Increased SID of Pro, Leu, and Glu in LSBM diets may be related to dietary N. Zhai and Adeola (2011) reported a negative linear relationship between the digestibility of several AA and dietary CP, with SID of AA decreasing as CP increased. Included in this were Leu and Glu, and although not significant, Pro decreased as well. Decreases may be related to an oversupply of AA in the HSBM diet that would saturate AA transporters in the small intestine.

Hindgut Disappearance

Hindgut disappearance was calculated from AID and ATTD values for all pigs at 5-8 and 16-19 dpi to determine differences attributed to diet, PRRS, or their interaction (Table 2.9). No diet × PRRS interaction was detected at either collection period. At 5-8 dpi, PRRS increased ($P < 0.03$) hindgut disappearance of DM and GE by 21% and 23%, respectively in pigs fed a complete diet. Interestingly, there was a tendency ($P = 0.10$) for PRRSV infection to lead to reduced DM disappearance in the hindgut at 16-19 dpi. The increase in hindgut DM and GE disappearance is likely related to an increase in microbial density in the cecum and colon of PRRSV challenged pigs (Niederwerder et al., 2016).

Diet significantly ($P < 0.001$) influenced all parameters at 5-8 dpi. In pigs fed HSBM diets, hindgut disappearance of DM, N, and GE were all increased compared to pigs fed LSBM diets. Similarly, at 16-19 dpi, DM disappearance was significantly increased ($P = 0.023$) while N and GE disappearance tended to be increased ($P = 0.082$ and $P = 0.051$, respectively) in pigs fed HSBM diet. Increased disappearance of nutrients and energy in the hindgut of pigs fed HSBM diets was likely due to the increased CP content in the diet, and therefore, increased protein reaching the cecum and colon promoting microbial growth. Although pigs cannot readily absorb and utilize N from the hindgut for protein deposition (Rérat, 1978), energy used by the hindgut can contribute to maintenance energy and improve feed efficiency (Dierick et al., 1990).

Hindgut disappearance was also determined in the N-free pigs to determine differences between control and PRRSV challenged pigs. Surprisingly, no differences ($P > 0.10$) were detected at 5-8 or 16-19 dpi. A numerical increase in DM disappearance at both collection periods (62 and 55%, respectively) was seen in PRRSV pigs compared to control pigs; however, due to high variation, no significance was detected. A potential increase in DM disappearance

coupled with increased total tract endogenous N loss could be a result of increased microbial activity and/or abundance. In pigs fed protein-free diets, fecal and microbial protein composition is similar (Taverner et al., 1981), likely indicating an increase in microbial abundance in the hindgut of PRRSV pigs which can have a beneficial outcome (Niederwerder et al., 2016).

Conclusion

Diet is known to impact AID and SID of AA. Crystalline AA are assumed to be 100% digestible, so when a diet is supplemented with crystalline AA, digestibility increases as demonstrated in the current study. Similarly, as dietary AA content decreases, AID and SID increase (Otto et al., 2003). Diet also can alter the microbial profile in the gut leading to changes in hindgut disappearance of nutrients. Health challenges are known to impact AID but studies to determine SID values are scarce. Digestibility of AA during health challenge appears to be dependent on the stage of disease. After 24 h of *Salmonella* Typhimurium, AID of AA were minimally impacted while after 72 h, AID of Lys, Phe, Thr, and Ser were reduced (Lee, 2012). Interestingly, SID of His and Gly were increased at 24 h and SID of Lys was reduced at 72 h. In the current study, only SID of Arg, Gly, and Pro at 7-8 dpi and SID of Pro at 18-19 dpi were reduced. Similarly, environmental stress and pathogens impact BEL of AA where it has been shown that heat stressing pigs for 2 d resulted in increased BEL of Arg and His. After 8 d of adaptation to heat stress, BEL of total nonessential AA and Pro increased by 16 and 54%, respectively. In contrast, nursery or grower pigs challenged with *Salmonella* Typhimurium demonstrated increased BEL of all AA within 24 h but were not different after 56 h (Lee, 2012). In the current study, PRRSV reduced BEL of Arg, Ala, and Pro at 7-8 dpi only. Although oppositely affected, BEL differences were detected around peak disease in these studies and were not different during the recovery phase.

Altogether, these data suggest that potential benefits of feeding increased SBM during a PRRSV challenge are likely not related to digestibility of nutrients or AA. Also, PRRSV has little impact on digestibility. In contrast to other challenge models, BEL of some AA were reduced at peak viremia and were not different during seroconversion, although there is high variability associated with the determination of these values. In conclusion, SBM inclusion impacts SID of AA and hindgut disappearance of nutrients, regardless of PRRSV. Further, there was minimal impact of PRRSV on BEL, and therefore, SID of most AA were not different.

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Table 2.1. Diet composition, as-fed basis

Ingredient, %	**HSBM**	**LSBM**	**N-free**
Corn	67.22	83.90	-
Cornstarch	-	-	78.95
Soybean meal, 46.5% CP	29.72	10.00	-
Dextrose	-	-	10.00
Solka floc	-	-	4.00
Soybean oil	-	-	3.00
Casein	-	2.17	-
Monocalcium phosphate	0.79	0.85	1.35
Limestone	0.97	1.09	1.00
Salt	0.50	0.50	0.50
L-Lys·HCl	-	0.43	-
L-Thr	-	0.13	-
L-Trp	-	0.03	-
Chromic oxide	0.40	0.40	0.40
Potassium carbonate	-	-	0.40
Vitamin premix[1]	0.15	0.15	0.15
Mineral premix[2]	0.15	0.15	0.15
Magnesium oxide	-	-	0.10

HSBM=high soybean meal; LSBM=low soybean meal.
[1]Provided per kilogram of diet: 6,125 IU vitamin A, 700 IU vitamin D_3, 50 IU vitamin E, 30 mg vitamin K, 0.05 mg vitamin B_{12}, 11 mg riboflavin, 56 mg niacin, and 27 mg pantothenic acid.
[2]Provided per kilogram of diet: 22 mg Cu (as $CuSO_4$), 220 mg Fe (as $FeSO_4$), 0.4 mg I (as $Ca(IO_3)_2$), 52 mg Mn (as $MnSO_4$), 220 mg Zn (as $ZnSO_4$), and 0.4 mg Se (as Na_2SeO_3).

Table 2.2. Calculated and analyzed nutrient composition of experimental diets, as-fed basis

	Calculated			Analyzed		
Parameter	**HSBM**	**LSBM**	**N-free**	**HSBM**	**LSBM**	**N-free**
DM, %	89.2	89.5	-	94.6	94.6	96.3
Energy, Mcal/kg[1]	3.31	3.33	3.71	4.00	3.88	3.82
CP, %	19.4	14.2	0.20	18.5	13.0	0.73
Indispensable AA, %						
Arg	1.17	0.66	0.01	1.15	0.60	0.01
His	0.48	0.34	0.01	0.49	0.33	0.02
Ile	0.72	0.48	0.01	0.80	0.51	0.02
Leu	1.51	1.26	0.03	1.60	1.20	0.05
Lys	0.92	0.92	0.00	1.12	1.10	0.03
Met	0.28	0.31	0.00	0.27	0.29	0.01
Met + Cys	0.55	0.52	0.00	0.55	0.47	0.10
Phe	0.85	0.60	0.01	0.94	0.62	0.02
Thr	0.61	0.56	0.01	0.71	0.60	0.01
Trp	0.21	0.16	0.00	0.19	0.14	0.00
Val	0.79	0.60	0.01	0.89	0.61	0.02
Dispensable AA, %						
Ala	-	-	-	0.93	0.65	0.03
Asp	-	-	-	1.90	1.05	0.03
Cys	0.27	0.21	0.00	0.28	0.18	0.09
Glu	-	-	-	3.28	2.26	0.06
Gly	-	-	-	0.77	0.45	0.01
Pro	-	-	-	1.03	0.90	0.03
Ser	-	-	-	0.81	0.54	0.02
Tyr	0.55	0.45	0.01	0.50	0.29	0.01

HSBM = high soybean meal; LSBM = low soybean meal; N-free = nitrogen-free.
[1]Calculated composition = Mcal ME/kg; analyzed composition = Mcal GE/kg.

Table 2.3. PRRS viremia and antibody titer of pigs fed high and low soybean meal diets or nitrogen-free diet during PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						Diet	*dpi*	*Diet×dpi*
PRRS viremia (PCR Ct value)[2]								
7 dpi	≥37	20.1	≥37	19.5	1.29	0.205	<0.001	0.567
14 dpi	≥37	27.3	≥37	24.7				
21 dpi	≥37	32.6	≥37	30.6				
PRRSX3 antibody (S/P ratio)[3]								
7 dpi	<0.40	0.97	<0.40	0.97	0.36	0.550	<0.001	0.400
14 dpi	<0.40	2.42	<0.40	2.27				
21 dpi	<0.40	2.04	<0.40	2.57				

Nitrogen-free diet	Control	PRRS	SEM	*P*-value[1]		
				Diet	*dpi*	*Diet×dpi*
PRRS viremia (PCR Ct value)[2]						
7 dpi	≥37	19.3	0.91	<0.001	<0.001	<0.001
14 dpi	≥37	26.2	1.08			
21 dpi	≥37	32.4	1.08			
PRRSX3 antibody (S/P ratio)[3]						
7 dpi	<0.40	0.94	0.01	<0.001	<0.001	<0.001
14 dpi	<0.40	2.03	0.13			
21 dpi	<0.40	2.17	0.13			

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
[1]main effect of diet, day post inoculation (dpi), and interaction of diet × dpi.
[2]Ct ≥37 denotes negative PRRS outcome.
[3]PRRSX3 antibody S/P ratio <0.40 denotes PRRS negative.

Table 2.4. Apparent total tract and ileal digestibility coefficients (%) in pigs fed high and low soybean meal diets at 5 to 8 dpi PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						PRRS	*Diet*	*PRRS×Diet*
ATTD[2], %								
DM	88.52	87.92	87.32	87.83	3.30	0.930	0.220	0.283
N	85.91	85.55	81.65	80.35	4.14	0.533	0.002	0.720
GE	86.58	86.23	84.14	84.55	3.12	0.958	0.004	0.550
AID[3], %								
DM	72.08	66.01	76.58	74.15	3.43	0.030	0.003	0.327
N	77.49	73.87	76.65	76.00	3.16	0.220	0.706	0.388
GE	73.02	67.39	75.98	73.38	3.87	0.040	0.027	0.425
Indispensable AA, %								
Arg	85.91	84.14	80.81	83.07	2.28	0.812	0.007	0.063
His	83.52	81.17	79.16	81.17	2.24	0.910	0.163	0.163
Ile	80.75	80.78	78.45	79.66	2.07	0.574	0.134	0.594
Leu	82.21	81.85	81.75	83.47	1.95	0.527	0.589	0.340
Lys	82.57	85.12	87.52	88.34	1.18	0.170	0.003	0.475
Met	84.11	85.54	88.14	89.55	1.37	0.138	<0.001	0.995
Phe	81.91	81.68	80.50	81.80	1.99	0.615	0.548	0.480
Thr	76.72	70.90	78.76	77.82	2.66	0.099	0.033	0.223
Trp	81.00	78.60	82.17	80.82	1.98	0.266	0.311	0.752
Val	76.87	76.04	74.66	75.42	2.29	0.984	0.321	0.573
Dispensable AA, %								
Ala	78.40	79.41	76.71	78.30	2.06	0.335	0.303	0.827
Asp	81.87	82.36	79.15	79.57	1.45	0.688	0.023	0.975
Cys	75.35	70.17	69.27	69.73	3.68	0.270	0.134	0.190
Glu	85.09	84.88	84.32	86.20	1.60	0.368	0.766	0.262
Gly	67.37	62.80	57.79	59.71	4.78	0.603	0.020	0.206
Pro	73.51	75.30	81.58	82.57	3.47	0.644	0.022	0.894
Ser	81.57	78.41	79.00	79.15	1.50	0.327	0.547	0.283
Tyr	79.14	76.82	74.37	75.55	2.86	0.716	0.067	0.272

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
dpi = days post inoculation.
[1]main effect of diet, PRRS, and interaction of PRRS × diet.
[2]ATTD = apparent total tract digestibility.
[3]AID = apparent ileal digestibility.

Table 2.5. Apparent total tract and ileal digestibility coefficients (%) in pigs fed high and low soybean meal diets at 16 to 19 dpi PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						PRRS	*Diet*	*PRRS×Diet*
ATTD[2], %								
DM	86.01	85.22	86.05	85.51	1.32	0.239	0.762	0.825
N	84.55	83.85	80.23	82.02	1.41	0.670	0.024	0.325
GE	84.92	84.06	83.77	83.85	1.79	0.557	0.312	0.477
AID[3], %								
DM	67.85	68.95	74.90	71.76	4.90	0.635	0.033	0.329
N	74.09	75.69	72.98	73.35	5.03	0.650	0.433	0.775
GE	69.32	71.00	74.84	72.19	4.69	0.826	0.141	0.330
Indispensable AA, %								
Arg	85.05	86.75	79.69	82.06	3.74	0.227	0.008	0.840
His	82.95	83.76	79.09	81.98	4.88	0.277	0.112	0.539
Ile	79.02	79.99	79.17	78.01	3.16	0.952	0.574	0.497
Leu	80.68	81.45	80.94	81.91	3.91	0.606	0.831	0.951
Lys	82.75	83.14	86.11	86.85	5.30	0.740	0.052	0.918
Met	83.70	85.29	87.38	88.54	2.97	0.324	0.023	0.876
Phe	80.34	81.09	81.19	80.94	3.07	0.860	0.815	0.729
Thr	74.61	73.93	76.07	76.68	4.66	0.987	0.312	0.750
Trp	79.44	79.57	80.22	82.57	3.29	0.392	0.208	0.448
Val	74.21	75.64	71.66	73.16	4.90	0.521	0.286	0.987
Dispensable AA, %								
Ala	75.58	77.71	77.14	76.19	3.23	0.748	0.992	0.404
Asp	79.63	79.71	76.07	77.68	4.57	0.657	0.161	0.689
Cys	73.85	71.68	67.37	69.02	6.44	0.916	0.081	0.444
Glu	83.72	81.40	83.21	83.46	4.39	0.518	0.636	0.431
Gly	63.53	66.24	55.49	57.21	8.37	0.485	0.017	0.875
Pro	72.37	75.14	75.89	81.66	4.60	0.300	0.230	0.724
Ser	80.86	81.12	77.38	77.47	3.42	0.915	0.043	0.958
Tyr	78.28	78.50	74.71	74.85	3.35	0.916	0.057	0.980

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
dpi = days post inoculation.
[1]main effect of diet, PRRS, and interaction of PRRS × diet.
[2]ATTD = apparent total tract digestibility.
[3]AID = apparent ileal digestibility.

Table 2.6. Basal endogenous loss of N and AA (g/kg DMI) due to PRRSV infection

Parameter	7-8 dpi				18-19 dpi			
	Control	**PRRS**	**SEM**	***P*-value**	**Control**	**PRRS**	**SEM**	***P*-value**
Fecal N	2.50	3.44	1.71	<0.001	2.83	3.05	0.70	0.637
Ileal N	3.43	2.21	1.22	0.087	4.05	2.46	1.54	0.302
Indispensable AA								
Arg	0.90	0.32	0.30	0.022	1.18	0.42	0.53	0.214
His	0.25	0.22	0.08	0.587	0.21	0.19	0.09	0.736
Ile	0.41	0.34	0.16	0.408	0.33	0.36	0.14	0.730
Leu	0.66	0.64	0.27	0.876	0.52	0.63	0.26	0.437
Lys	0.74	0.45	0.27	0.131	0.56	0.56	0.35	0.971
Met	0.11	0.09	0.05	0.406	0.08	0.10	0.04	0.643
Phe	0.41	0.39	0.17	0.841	0.33	0.41	0.17	0.363
Thr	0.64	0.72	0.24	0.482	0.49	0.77	0.25	0.057
Trp	0.13	0.14	0.05	0.627	0.10	0.13	0.05	0.287
Val	0.63	0.59	0.23	0.745	0.51	0.63	0.21	0.323
Dispensable AA								
Ala	0.75	0.46	0.29	0.050	0.79	0.48	0.36	0.329
Asp	0.98	0.78	0.37	0.314	0.82	0.85	0.38	0.888
Cys	0.28	0.25	0.10	0.620	0.19	0.23	0.09	0.171
Glu	1.21	0.96	0.51	0.335	1.00	0.99	0.46	0.970
Gly	1.92	1.37	0.68	0.310	2.48	1.38	1.05	0.264
Pro	7.59	0.43	2.13	0.009	8.17	3.51	2.29	0.188
Ser	0.61	0.51	0.19	0.299	0.50	0.52	0.20	0.764
Tyr	0.29	0.26	0.11	0.650	0.23	0.26	0.10	0.657

dpi = days post inoculation.

Table 2.7. Standardized ileal digestibility coefficients (%) in pigs fed high and low soybean meal diets at 7 to 8 dpi PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						PRRS	*Diet*	*PRRS×Diet*
N	78.15	74.30	77.27	76.41	3.16	0.177	0.718	0.386
Indispensable AA, %								
Arg	93.44	88.01	95.12	88.24	1.89	<0.001	0.317	0.448
His	88.67	88.44	86.62	87.84	1.62	0.724	0.354	0.606
Ile	85.56	84.80	85.99	85.97	2.07	0.728	0.475	0.740
Leu	86.08	85.61	86.92	88.49	1.95	0.609	0.096	0.347
Lys	88.79	88.95	93.85	92.25	1.18	0.548	0.002	0.463
Met	87.96	88.67	91.72	92.47	1.37	0.437	0.001	0.985
Phe	86.01	85.62	86.72	87.78	1.99	0.752	0.192	0.501
Thr	85.24	80.50	88.85	89.18	2.66	0.269	0.005	0.206
Trp	87.59	85.77	91.12	90.55	1.98	0.472	0.020	0.707
Val	83.58	82.36	84.46	84.64	2.29	0.712	0.271	0.618
Dispensable AA, %								
Ala	86.06	84.06	87.68	84.95	2.06	0.090	0.354	0.788
Asp	86.77	86.26	88.02	86.63	1.45	0.405	0.476	0.698
Cys	84.67	78.55	83.76	82.78	3.68	0.104	0.434	0.232
Glu	88.59	87.66	89.40	90.23	1.60	0.955	0.077	0.341
Gly	90.15	78.26	97.32	81.65	7.56	0.002	0.188	0.630
Pro	137.49	77.89	160.59	86.34	5.05	<0.001	<0.001	0.061
Ser	88.66	84.31	89.63	88.00	1.50	0.060	0.136	0.376
Tyr	84.64	81.83	83.86	84.19	2.86	0.433	0.616	0.322

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
dpi = days post inoculation.
[1]main effect of diet, PRRS, and interaction of PRRS × diet.

Table 2.8. Standardized ileal digestibility coefficients (%) in pigs fed high and low soybean meal diets at 18 to 19 dpi PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						PRRS	*Diet*	*PRRS×Diet*
N	87.49	86.08	91.18	88.70	3.29	0.408	0.201	0.820
Indispensable AA, %								
Arg	95.35	93.33	97.79	92.98	2.34	0.133	0.656	0.538
His	87.17	87.62	87.26	88.18	3.79	0.602	0.812	0.856
Ile	82.87	84.02	84.75	84.46	3.23	0.789	0.492	0.653
Leu	83.73	84.84	84.82	86.62	4.04	0.387	0.403	0.838
Lys	87.51	87.86	90.95	91.64	5.28	0.758	0.047	0.917
Met	86.62	88.50	90.12	91.58	3.08	0.232	0.029	0.882
Phe	83.43	84.80	83.60	86.39	4.05	0.234	0.615	0.684
Thr	80.90	82.27	83.22	87.31	5.54	0.205	0.100	0.524
Trp	84.33	85.24	86.55	90.65	3.62	0.119	0.025	0.312
Val	79.55	81.69	79.03	82.39	5.15	0.239	0.969	0.791
Dispensable AA, %								
Ala	83.68	84.20	85.53	84.18	3.51	0.859	0.702	0.692
Asp	83.73	83.86	82.95	85.33	4.51	0.517	0.861	0.564
Cys	80.10	78.69	76.51	80.41	6.71	0.619	0.714	0.300
Glu	86.62	84.27	87.21	87.65	4.35	0.554	0.237	0.395
Gly	94.67	89.90	100.26	92.88	6.48	0.193	0.367	0.777
Pro	152.21	127.07	163.17	134.13	11.54	0.001	0.189	0.774
Ser	86.65	87.08	85.58	86.56	3.43	0.670	0.641	0.868
Tyr	82.69	83.17	81.66	83.13	3.47	0.589	0.776	0.786

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
dpi = days post inoculation.
[1]main effect of diet, PRRS, and interaction of PRRS × diet.

Table 2.9. Hindgut disappearance of nutrients and energy in pigs fed high and low soybean meal diets after PRRSV infection

Parameter	HSBM-	HSBM+	LSBM-	LSBM+	SEM	*P*-value[1]		
						PRRS	*Diet*	*PRRS×Diet*
Complete diet								
5-8 dpi								
DM, g/d	244	312	158	197	19.7	0.014	<0.001	0.462
N, g/d	3.93	4.94	1.22	1.28	0.83	0.293	<0.001	0.353
GE, Mcal/d	0.84	1.05	0.48	0.65	0.08	0.026	<0.001	0.771
16-19 dpi								
DM, g/d	286	219	196	172	50.1	0.100	0.023	0.417
N, g/d	4.89	2.79	1.45	1.96	1.43	0.491	0.082	0.266
GE, Mcal/d	1.01	0.70	0.64	0.56	0.19	0.114	0.051	0.339
Nitrogen-free diet	**Control**	**PRRS**	**SEM**	***P*-value**				
5-8 dpi								
DM, g/d	57	151	40.8	0.129				
N, g/d	0.97	-0.66	1.17	0.345				
GE, Mcal/d	0.18	0.23	0.13	0.787				
16-19 dpi								
DM, g/d	44	98	41.0	0.374				
N, g/d	1.96	1.46	1.57	0.824				
GE, Mcal/d	0.12	0.27	0.17	0.523				

HSBM-, LSBM- = high soybean meal (HSBM), low soybean meal (LSBM) without PRRS; HSBM+, LSBM+ = high soybean meal (HSBM), low soybean meal (LSBM) with PRRS.
dpi = days post inoculation.
[1]main effect of diet, PRRS, and interaction of PRRS × diet.

CHAPTER 3. IMPACT OF *BRACHYSPIRA HYODYSENTERIAE* ON INTESTINAL AMINO ACID DIGESTIBILITY AND ENDOGENOUS AMINO ACID LOSSES IN PIGS

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Abstract

Brachyspira hyodysenteriae (Bhyo) induces mucohemorrhagic diarrhea and colitis and is an economically significant disease in grow-finish pigs worldwide. Our objectives were to determine the impact of Bhyo on apparent total tract digestibility (ATTD), ileal digestibility (AID), and ileal basal endogenous losses (BEL) in grower pigs. In addition, we assessed the effect of Bhyo on hindgut disappearance of DM, N, and GE. Thirty-two Bhyo negative gilts (38.6 ± 0.70 kg BW) were fitted with a T-cannula in the distal ileum. Over two replicates, pigs were fed a complete diet (7 control, 10 Bhyo pigs) or nitrogen-free diet (NFD; 4 control, 11

Bhyo pigs). The 21 Bhyo pigs (62.6 ± 1.39 kg BW) were inoculated with Bhyo on day post inoculation (dpi) 0, and the 11 control pigs were sham inoculated. Feces were collected from 9 to 11 dpi and ileal digesta collected from 12 to 13 dpi. All pigs were euthanized at 14 to 15 dpi and intestinal tract pathology assessed. Feed, feces, and digesta were analyzed for DM, N, and GE. Feed and digesta were analyzed for AA. Within the complete diet and NFD treatments, data were analyzed to determine pathogen effects. All control pigs remained Bhyo negative, and 5 challenged pigs in each replicate were confirmed Bhyo positive within 9 dpi. Infection with Bhyo reduced ATTD of DM, N, and GE and increased AID of Gly ($P < 0.05$). No other AA AID differences were observed. Only BEL of Pro was reduced ($P < 0.05$) while Arg, Trp, and Gly tended ($P < 0.10$) to be reduced by Bhyo infection. When calculated from AID and BEL, Bhyo infection reduced SID of N, Arg, Lys, Ala, Gly, Pro, and Ser ($P < 0.05$) and tended to reduce Thr SID ($P = 0.09$). In the hindgut of Bhyo pigs, there was generally an appearance of nutrients rather than disappearance. In pigs fed a complete diet, hindgut appearance of N and GE were increased ($P < 0.05$) by 58 and nine-fold, respectively, and DM tended to be increased two-fold ($P = 0.06$). Similarly, in NFD fed pigs, hindgut appearance of N and GE was increased by 172 and 162%, respectively, although high variability led to no significance. Altogether, Bhyo infection has minimal impact on AID or BEL of AA; however, SID of N, Arg, Lys and some nonessential AA are reduced. This may suggest an increased need for AA and energy during a Bhyo challenge.

KEYWORDS: amino acid, digestibility, endogenous loss, pig, *Brachyspira hyodysenteriae*

Introduction

Brachyspira hyodysenteriae (**Bhyo**), the classical agent of swine dysentery (**SD**), affects pigs worldwide and is a reemerging pathogen in U.S. swine (Burrough, 2017). Although infection is more prominent in finishing pigs, younger pigs can experience disease with mortality and morbidity approaching 30 and 90%, respectively (Hampson, 2012). Coupled with decreased growth performance (Wilberts et al., 2014a), Bhyo causes considerable economic loss worldwide (Hampson, 2012). The hallmark clinical sign of Bhyo infection is mucohemorrhagic diarrhea which generally appears within 14 days of experimental infection (Kinyon et al., 1977; Stanton, 2006). Lesions from Bhyo occur in the cecum and colon and are characterized by hemorrhages and necrosis (Albassam et al., 1985; Quintana-Hayashi et al., 2015); however, the small intestine remains clinically unaffected (Stanton, 2006). Interestingly, little is known about how Bhyo infection modulates digestive tract function and nutrient and energy digestibility.

When animals are infected by different pathogens, tissue accretion rates and feed efficiency are reduced (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017; Helm et al., 2018), suggesting a repartitioning or reallocation of nutrients away from growth to support immune activation (Rauw, 2012) and barrier defense mechanisms such as mucus production (Colditz, 2008). However, how these insults alter digestibility and endogenous losses of AA and energy in the intestinal tract, and thus essential AA usage and requirements, is poorly understood. In nursery pigs, porcine epidemic diarrhea virus (**PEDV**) reduced apparent total tract digestibility (**ATTD**) of nutrients and energy; however apparent ileal digestibility (**AID**) of nutrients, energy, and AA, except Lys, were unaffected (Schweer et al., 2016). We have also shown that porcine reproductive and respiratory syndrome virus (**PRRSV**) reduces basal endogenous loss (**BEL**) of Arg and Ala at dpi 7 to 8 and BEL of Pro at 7 to 8 and 18 to 19 dpi

(Chapter 2). In contrast, Lee (2012) reported a reduction in AID of several AA and an increase in BEL of all AA within 24 h after *Salmonella* Typhimurium infection. Thus, it is assumed that enteric challenges such as SD may increase BEL of AA in the intestinal tract and this would alter SID values for AA.

Furthermore, AA metabolism is likely altered due to SD. In pigs that developed clinical SD, glucose metabolism, likely through glycolysis, was increased (Somchit et al., 2003). Similarly, Jonasson et al. (2007) reported a decrease in serum concentrations of gluconeogenic AA during clinical SD. Further, Wilberts et al. (2014b) and Quintana-Hayashi et al. (2015) have reported an increase in colonic mucin secretion in relation to Bhyo infection and considering mucins are rich in Thr, Ser, and Cys (Lien et al., 1997), dietary supply and metabolism of these AA may alter mucin synthesis and BEL. Therefore, the objective of this study was to determine how Bhyo modulated the digestibility of nutrients, energy, and AA, and to determine BEL of AA in response to Bhyo infection. We hypothesized that Bhyo would decrease AID and ATTD coefficients and increase BEL of AA.

Materials and Methods

All animal work was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 1-16-8156-S) and adhered to the ethical and humane use of animals for research.

The experiment was performed in two replicates consisting of 17 and 15 pigs, respectively. In all, 32 gilts (38.6 ± 0.70 kg BW) were selected and surgically fitted with a T-cannula in the distal ileum as described by Stein et al. (1998). Thirty-five days after surgery, and following a PRRSV study (Chapter 2), pigs were utilized for this Bhyo challenge study. Pigs

were individually penned (1.4 × 1.5 m) across two rooms. Because Bhyo is spread through feces and is not aerosolized, there were control (non-challenged) and Bhyo challenged pigs in each room with approximately 7 m separating control and Bhyo pens. In total, there were 11 control pigs (7 and 4 in replicate 1 and 2, respectively) and 21 Bhyo (10 and 11 in replicate 1 and 2, respectively) pigs across both replicates. All pigs were weighed on dpi 0 and at the end of the challenge period (dpi 14 to 15).

Animal Inoculation, Clinical Evaluation, and *Brachyspira* Detection

Fresh rectal swabs were collected from all animals immediately prior to the start of each replicate and were submitted for selective anaerobic culture targeting *Brachyspira* spp. at the Iowa State University Veterinary Diagnostic Laboratory (**ISU VDL**) to confirm negative status. At 62.6 ± 1.39 kg BW, on day post inoculation (**dpi**) 0 and 2, the 21 Bhyo challenged pigs were inoculated with 30 mL of agar slurry containing *Brachyspira hyodysenteriae* B204 via their terminal ileum T-cannula. The agar slurry was prepared as previously described (Burrough et al., 2012) and the inoculation dose was approximately 1.4×10^6 CFU/mL on dpi 0 and 1.2×10^5 CFU/mL on dpi 2. The control pigs received a sham inoculation with 30 mL of sterile agar intra-cannularly on dpi 0 and 2.

Pigs were examined daily for the development of diarrhea. Feces were examined and scored 0 if normal, 1 if semi-formed, 2 if semi-liquid, 3 if watery or mucoid +/- blood [mucoid to mucohemorrhagic diarrhea (**MD**)].

To detect Bhyo infection and shedding, in replicate one, rectal swabs were taken for culture on dpi 5, 7, 9, 12, and at necropsy (dpi 14 or 15). In the second replicate, PCR was performed on feces collected at dpi 9 and 12, and selective culture was performed on feces at necropsy (dpi 14 or 15). Freshly collected samples were submitted to the ISU VDL and were

processed routinely for *Brachyspira* selective culture and/or PCR using standard methods of the laboratory.

Pathology and Histopathology

At dpi 14 to 15, pigs were euthanized by captive bolt followed by exsanguination and a necropsy was performed to assess gross lesions and to collect samples for histopathology. Specifically, the lungs were examined for lesions related to the prior PRRSV infection, and the jejunum, ileum, cecum, and colon were examined for gross lesions of enteric disease. Sections of lung, distal ileum, cecum, spiral colon (apex), and descending colon were fixed in 10% neutral buffered formalin and were processed routinely at the ISU VDL for histopathology. All tissues were examined after hematoxylin and eosin staining, and sections of spiral colon were also examined following Warthin-Starry silver staining to detect spirochetes.

For histopathology, sections were semi-quantitatively scored by a pathologist blinded to the treatment groups using the following guidelines. Sections of lung were evaluated for lesions of pneumonia and scored 0 if no significant lesions, 1 if mild lymphohistiocytic interstitial pneumonia, 2 if moderate lymphohistiocytic interstitial pneumonia, and 3 if moderate lymphohistiocytic interstitial pneumonia and neutrophilic alveolar infiltration. Sections of ileum were examined for the presence or absence of proliferative and/or neutrophilic ileitis typical of *Lawsonia* or *Salmonella* infection. Sections of cecum, spiral colon, and descending colon were each examined independently and scored 0 if no significant lesions; 1 if there was mild, diffuse mucosal thickening and lymphoplasmacytic infiltration of the lamina propria; 2 if there was moderate, diffuse mucosal thickening with neutrophilic infiltration and mucus-filled crypts; and 3 if moderate to severe diffuse mucosal thickening with a combination of neutrophil infiltration, neutrophilic exudation, superficial hemorrhage, crypt ectasia, and luminal accumulations of

mucus and erythrocytes. The semi-quantitative scores from the cecum, spiral colon, and descending colon were then combined to form a composite colitis score (**CC**) for each pig that was then used for statistical analysis. Sections of spiral colon that had been silver-stained were examined for the presence or absence of spirochetes with features typical of *Brachyspira* spp.

Diets and Feeding

In the first replicate, all pigs were fed a complete corn-soybean meal based diet (Table 3.1) formulated to meet or exceed nutrient and energy requirements (NRC, 2012). In the second replicate, all pigs were fed the complete diet and a nitrogen-free diet (**NFD**; formulated based on cornstarch; Table 3.1), during the two-week study period to determine BEL associated with Bhyo (4 control, 11 Bhyo pigs). The NFD was fed from 5 dpi through 13 dpi and pigs were fed the complete diet otherwise. Both diets contained 0.40% chromic oxide (**Cr_2O_3**) as an indigestible marker. All pigs were restrictively fed to ensure the entire meal was eaten during the collection period. Based on pig BW, pigs were fed 2.5 times the estimated energy requirement for maintenance (2.5 × 197 kcal of ME per $BW^{0.60}$; NRC, 2012).

Digesta And Fecal Sample Collection, Analysis, and Calculations

A representative feed sample from the complete diet and NFD were obtained for analysis. In the first replicate, fecal collections started when approximately 50% of pigs started to exhibit clinical signs (loose, watery stool; ~dpi 9) of Bhyo and ileal digesta collections followed. In both replicates, feces were collected from 9 to 11 dpi and pooled by pig. Ileal digesta was collected from 0800 to 1600 h on 12 and 13 dpi. Digesta was collected by attaching a 207 mL plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed when filled with digesta or every 30 min, whichever occurred first. Fecal samples were

stored at -20°C until further analysis. Digesta samples were stored on dry ice in each room of the BSL2 facility during collections and transferred to -20°C after each collection day.

After the collection period, ileal and fecal samples were thawed and homogenized within pig. A subsample of digesta was collected, stored at -20°C, and lyophilized (Model 10-100; Virtis Co. Ltd., Gardiner, NY). Fecal samples were dried in a mechanical convection oven at 100°C. Feed, feces, and digesta samples were ground through a 1 mm screen (Model ZM1; Retsch Inc., Newton, PA) prior to analysis. Proximate analysis was performed on feed, feces, and ileal digesta samples as previously described (Chapter 2). Briefly, all samples were analyzed for DM (AOAC method 930.15), Cr_2O_3 according to Fenton and Fenton (1979), N using TruMac N (Leco Corporation, St. Joseph, MO), and GE using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Amino acid composition of feed and digesta samples was determined by the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) by cation-exchange HPLC (L8900 Amino Acid Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

After analysis of diet, feces, and digesta components, the AID and ATTD were calculated using the following equation (Stein et al., 2007):

AID or ATTD = 100 – [100 × (concentration of Cr_2O_3 in diet × concentration of component in feces or digesta ÷ concentration of Cr_2O_3 in feces or digesta × concentration of component in diet)]

The BEL of N and AA (g/kg DMI) were calculated using the equation (Stein et al., 2007):

BEL = [AA or N in digesta × (Cr_2O_3 in diet ÷ Cr_2O_3 in digesta)]

Standardized ileal digestibility values for N and AA were calculated by correcting AID values for BEL using the equation (Stein et al., 2007):

SID = [AID + (BEL ÷ AA in diet)]

The study design was not a crossover; therefore, each pig could not serve as its own control for SID determination. Consequently, statistical analysis was performed on BEL values from the second replicate, and the reported treatment averages were applied to AID values from the first replicate to determine SID values.

Hindgut disappearance of DM (g/d), N (g/d), and GE (Mcal/d) were calculated using the following equation:

Hindgut disappearance = Amount at terminal ileum – Amount excreted in feces

Statistical Analysis

Data were initially analyzed to determine if there was an effect of PRRSV from the prior study or if there was an interaction between PRRSV and Bhyo challenge on any parameters. There was no PRRSV effect or interaction between PRRSV and Bhyo challenge for any parameters assessed ($P > 0.10$); therefore, data were analyzed to determine the main effect of Bhyo challenge. Digestibility, BEL, and hindgut disappearance data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc. Cary, NC). Data from control and Bhyo pigs fed a complete diet were analyzed to determine treatment effects on AID, SID, and hindgut disappearance of nutrients and energy. Data from control and Bhyo pigs fed NFD were analyzed separately from the pigs fed the complete diet to determine the impact of Bhyo on BEL and hindgut disappearance of nutrients and energy. The CC score was analyzed using the FREQ procedure with the Fisher's exact test to assess the effect of Bhyo challenge on lesion score distribution. Pig was considered the experimental unit for all analyses. All digestibility, BEL,

and hindgut disappearance data are reported as least squares means ± SEM and considered significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

Results and Discussion

Under the resource allocation theory (Rauw, 2012), it is anticipated that nutrient requirements, specifically AA, are altered during times of disease and stress due to nutrient repartitioning away from growth in favor of the immune response (Humphrey and Klasing, 2004). Further, this also extends to potential increases in BEL of AA due to increased intestinal mucin production and cell sloughing due to disease (Albassam et al., 1985; Lee, 2012; Wilberts et al., 2014b). In pigs, this notion of nutrient (i.e., AA) repartitioning is supported by research from our group, and others, where feed efficiency and tissue accretion rates are significantly reduced due to infection with systemic and enteric pathogen challenges (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017; Curry et al., 2018; Helm et al., 2018). However, it is unclear to what degree, if any, SID of AA requirements and BEL change during these disease states in growing pigs. We have previously shown that PRRSV challenge in grower pigs reduced BEL of Pro, Arg, and Ala during peak viremia, but only Pro BEL differed at seroconversion (Chapter 2). In similar size pigs, Lee (2012) reported increased BEL of several AA within 24 h of a *Salmonella* Typhimurium challenge; however, similar to PRRSV, BEL of AA returned to pre-inoculation values by 56 h post-inoculation. Surprisingly, given the global significance of Bhyo (Burrough, 2017), changes in nutrient utilization in pigs with Brachyspiral colitis remains unexplored. Therefore, the current experiment aimed to determine changes in nutrient, energy, and AA digestibility and quantify BEL of AA during a Bhyo challenge. This allowed for AA SID coefficients to be calculated and compared to SID values of healthy pigs (NRC, 2012).

Clinical Examination, *Brachyspira* Detection, and Pathology

All pigs were confirmed culture-negative for *Brachyspira* spp. prior to dpi 0 and the challenge model was confirmed via fecal PCR and culture for Bhyo (Table 3.3). Expectedly, control pigs in both replicates remained negative for Bhyo throughout the two-week study and clinical scores of feces were normal. In the first replicate, MD was first observed on dpi 8, and 50% (5/10) of the inoculated pigs were culture positive for Bhyo by dpi 9 when fecal collections began. At necropsy, 70% (7/10) of the Bhyo pigs were still culture positive, and two were exhibiting MD, suggesting full resolution had not occurred and there were persistent shedders. In the second replicate, MD was first observed on dpi 5, 4 pigs were exhibiting MD by dpi 8, and 45% (5/11) of inoculated pigs were positive for Bhyo by 9 dpi based on PCR. Interestingly, MD had completely resolved by dpi 10 in the second replicate, and at necropsy, only one pig was Bhyo positive by culture. These data suggest the NFD reduced the disease duration and helped to eliminate infection and shedding as the number of inoculated pigs still culture-positive at necropsy was reduced from 70% to 9%. Interestingly, histopathology assessment revealed that the distribution of CC scores was not significantly influenced by Bhyo challenge ($P = 0.258$; Table 3.3). In control pigs fed a complete diet or NFD did not have a CC score greater than 2; while 70% (7/10) and 27% (3/11) of Bhyo pigs fed complete or NFD, respectively, had a score of 3 or more. Further, Bhyo pigs fed a complete diet pigs had an increased incidence of severe scores (score of 6 to 9) compared to NFD fed, Bhyo challenged pigs (30% versus 0%, respectively). This further supports the protective effect of the NFD diet on SD expression in this study. Given that the NFD was based on cornstarch, and thereby highly digestible, this finding is

consistent with a previous experiment where feeding a highly digestible, rice-based diet was protective against Bhyo infection and SD expression (Pluske et al., 1996).

The relationship between diet and clinical presentation and shedding of *Brachyspira* has been explored with somewhat contradicting results. While there is general agreement that diet can significantly alter SD expression, the specific substrates responsible for these differences are not entirely clear. It is generally agreed that limiting fermentation in the hindgut reduces Bhyo colonization and SD expression (Siba et al., 1996; Pluske et al., 1998; Wilberts et al., 2014a); however, there is disagreement as to which types of fiber have the greatest impact with studies showing soluble fiber sources may increase disease (Pluske et al., 1998), soluble fiber sources such as inulin can decrease disease (Hansen et al., 2010; Hansen et al., 2011), and insoluble fibers sources such as lignins can increase SD expression (Wilberts et al., 2014a). Additionally, Durmic et al. (2000), Kirkwood et al. (2000), and Durmic et al. (2002) were unable to demonstrate the protective effect of reducing soluble non-starch polysaccharides or resistant starch content of the diets suggesting the dietary effect is likely multifactorial. Although a cornstarch based NFD is non-physiological and not commercially relevant, it further supports the notion that highly digestible and poorly fermentable diets are beneficial for resolution of Bhyo infection as the NFD contained minimal lignin or cellulose. Clinical Bhyo infection appears dependent on the microbiota populations in the large intestine, and the presence of other anaerobes, such as *Bacteroides vulgatus* and *Fusobacterium necrophorum* (Harris et al., 1978; Whipp et al., 1979), is required for SD expression. The colonic microbiota in the pig depends heavily on diet and is significantly altered by dietary fiber (Burrough et al., 2015), and although little is known about the impact of NFD on colonic microbial populations, it may provide a

potential mechanism for Bhyo resolution by altering the microbiota to a state that is unfavorable to Bhyo.

Although the inoculum was administered into a T-cannula positioned in the distal ileum in this study, progression of disease was not markedly different from that of pigs that have been orally inoculated with the same Bhyo isolate, where clinical signs are typically observed within 7 to 10 dpi (Kinyon et al., 1977; Wilberts et al., 2014a; Wilberts et al., 2014b). End BW was not different among treatments by dpi 14 to 15 (control pigs 67.6 ± 2.35 and 61.4 ± 4.20 kg BW, Bhyo pigs 69.0 ± 3.12 and 61.2 ± 2.13 kg BW, replicates one and two, respectively). Gross and microscopic lung lesions consistent with prior PRRSV infection were predominantly unapparent to mild (score 0 – 1) in both control and Bhyo pigs. A total of 4 animals had microscopic lung scores of 2, and a single animal scored a 3. The small intestines were grossly unremarkable and microscopic lesions were not observed in the sections of ileum examined.

Apparent Total Tract and Ileal Digestibility

The calculated and analyzed nutrient concentrations for the NFD and complete diet are presented in Table 3.2. Analyzed nutrient and energy composition of both diets were similar to calculated predicted values. At the start of fecal (dpi 9) and ileal collections (dpi 12), a similar number of pigs were Bhyo positive and had developed SD in both replicates; therefore, it was assumed that a similar number of pigs had been infected prior to the diet change (NFD at 5 dpi) and that the analyses for the two replicates could be combined. Apparent total tract digestibility of DM, N, and GE were assessed from 9 to 11 dpi (Table 3.4) by fecal grab sampling. The Bhyo challenge resulted in decreased DM, N, and GE ATTD coefficients by 3, 8, and 4%, respectively ($P < 0.02$, all parameters). These reductions in ATTD coefficients are likely related to diarrhea

and shortened passage time through the gut. The increased rate of passage can result in the insufficient enzymatic breakdown of feedstuffs leading to poor absorption (Blaxter and Wood, 1953; Bush et al., 1963). As a result, diarrhea scores have been negatively correlated with ATTD in pigs (Entringer et al., 1975). Limited data exist for the impact of enteric pathogens on ATTD, and there is no previous data on how Bhyo impacts small intestinal and total tract digestibility. We have previously reported that nursery pigs challenged with PEDV had reduced ATTD of DM, N, and GE compared to non-challenged controls (Schweer et al., 2016). Although in much younger pigs, these data are in agreement with the ATTD findings in the current study.

To assess the modulatory impact the Bhyo challenge may have in the small intestine, AID of DM, N, and GE were assessed from 12 to 13 dpi. Although there were differences in ATTD, no differences were noted for AID of DM, N, or GE over this period ($P > 0.10$, Table 3.4). These results are similar to a previous report from our group that reported no difference in AID of DM, N, or GE in 20 kg BW pigs challenged with PEDV (Schweer et al., 2016). However, when examining AA AID, an increase in the AID of Gly ($P = 0.039$) was the only difference observed (Table 3.4). This may not be surprising as the primary target of Bhyo is the cecum and colon, while small intestinal absorptive function may remain unaffected (Argenzio, 1980). Therefore, it is reasonable that the challenge did not alter AID coefficients while ATTD coefficients were reduced. This reduction in Gly AID may be a result of the need for Gly in purine and protein synthesis and the fact that Gly is readily metabolized by enterocytes (Wang et al., 2013; Wang et al., 2014). Glycine also serves a precursor for glutathione and can prevent oxidative stress and cytokine response which are induced during Brachyspiral challenges (Naresh et al., 2009; Chmielewska et al., 2013; Wang et al., 2014).

Basal Endogenous Losses

We hypothesized that there would be increases in total tract N and ileal AA losses due to the Bhyo challenge based on increased intestinal mucin secretion observed with Bhyo infection (Wilberts et al., 2014b). Additionally, these increases could also result from the presence of increased blood or sloughed cells in the feces of infected pigs. Therefore, a primary objective of this research was to determine if Bhyo challenge altered BEL of N or AA in growing pigs. Currently, BEL of N and AA are not well characterized in health compromised livestock species. As infection with Bhyo results in increased mucin secretion (Wilberts et al., 2014b; Quintana-Hayashi et al., 2015), it was anticipated that endogenous secretions of mucin related AA, namely Cys, Ser, and Thr, would increase. There was a 55% reduction in BEL of Pro ($P = 0.046$) and a tendency for BEL of Arg ($P = 0.092$), Trp ($P = 0.087$), Gly ($P = 0.096$), and total AA ($P = 0.081$) to be reduced due to Bhyo challenge (Table 3.5). However, the AA abundant in mucin (i.e., Cys, Ser, and Thr) were unaffected. In contrast, nursery and growing pigs challenged with *S.* Typhimurium had increased BEL of all AA within 24 h of infection (Lee, 2012). This discrepancy between pathogen challenges may be a result of pathogen site of colonization as *S.* Typhimurium can colonize both the small and large intestine (Côté et al., 2004), while Bhyo colonization is confined to the cecum and colon. Although total AA BEL tended to be reduced, BEL of N was numerically reduced compared to controls (4.40 vs. 2.71 g/kg DMI). Expectedly, total tract endogenous N loss was increased by Bhyo infection (4.89 vs. 1.03 g/kg DMI), although due to high variability because of small sample size, this was only numerically different. Reduced BEL and increased total tract N loss have been previously reported during a PRRSV infection (Chapter 2).

Although the use of NFD tends to result in overestimation of Pro and Gly BEL (Moughan et al., 1992), and when fed for extended periods Pro BEL increases (Jansman et al., 2002), BEL of Pro was drastically reduced due to Bhyo infection. Proline, its derivative hydroxyproline, and Gly are primary components of collagen (Eyre and Muir, 1975), which is involved in healing of the colonic wall after an insult (Hesp et al., 1984). Although Bhyo typically resides in the colonic crypts and luminal mucus, during severe infection colonocytes can become infected, detached, and slough away (Albassam et al., 1985), thus increasing the need for collagen for colonic wall reassembly. Arginine can also aid in healing of the colon (Shashidharan et al., 1999) and can improve antioxidant status (Ma et al., 2010), both of which are important for Bhyo resolution. Similarly, serotonin, a derivative of Trp, can mediate the stress response after infection by reducing glucocorticoid concentrations (Le Floc'h and Seve, 2007). Because increased glucocorticoid concentrations can stimulate Brachyspiral growth in culture (Naresh and Hampson, 2011), decreased BEL of Trp may likely be used for the synthesis of serotonin. Tryptophan is synthesized to serotonin in enterochromaffin cells. In chicks experimentally infected with Bhyo, the number of cecal enterochromaffin cells was not different (Sueyoshi and Adachi, 1990) suggesting no reduction in serotonin synthesis; however, data is unavailable for Bhyo infected pigs. Similarly, in patients with irritable bowel syndrome, tissue serotonin levels are increased (Faure et al., 2010), and *S.* Typhimurium infection increased luminal release of serotonin in the pig small intestine (Grøndahl et al., 1998).

Standardized Ileal Digestibility

Standardized ileal digestibility of AA was determined by correcting the AID values for BEL (Table 3.6). The SID of N, Arg, Lys, Ala, Gly, Pro, and Ser were reduced ($P < 0.05$) due to

Bhyo infection, and Thr SID tended to be reduced ($P = 0.088$). The greatest reductions occurred in SID of Pro and Gly, which were reduced by 32 and 16%, respectively. Few studies have determined BEL during infection in livestock species, and therefore, AA SID values during infection are scarce. As mentioned, BEL of some AA can be overestimated when using a NFD, leading to overestimation of some SID values. Although only BEL of Pro was significantly reduced, reductions in SID of N, Pro, Arg, Ala, and Gly are likely due to decreased BEL. Somewhat in agreement with the current study, we have reported in nursery pigs that PEDV reduced AID of Lys (Schweer et al., 2016); however, BEL was not determined, and SID could not be calculated. In PRRSV challenged grower pigs, SID of Arg, Gly, and Pro were also reduced, while Ala and Ser SID tended to be reduced during peak viremia (Chapter 2). This is similar to nursery pigs challenged with *S.* Typhimurium in which SID of Arg was reduced at 24 and 72 h post challenge, while SID of Gly was increased at 24 h post challenge (Lee, 2012). However, these enteric challenge SID results contradict the current study data presented herein (Table 3.6). Although, in agreement with our data, Lee (2012) reported in 8 and 24 h post challenge pigs that there were reductions in SID of several AA including Gly.

Hindgut Disappearance

Bhyo targets the cecum and colon resulting in mucohemorrhagic colitis and cell sloughing (Argenzio et al., 1980; Albassam et al., 1985). Therefore, it was anticipated that hindgut nutrient disappearance would decrease, resulting in a net nutrient appearance. Hindgut disappearance of DM, N, and GE was calculated from AID and ATTD values (Table 3.7). When pigs were fed a complete diet, Bhyo reduced the disappearance of N and GE ($P < 0.04$) and showed a strong tendency ($P = 0.055$) to reduce the disappearance of DM in the hindgut.

However, when pigs were fed a NFD, only numerical reductions in DM, N, and GE disappearance were observed and were reduced by 99, 172, and 162%, respectively, but due to small sample size causing high variation, differences were not significant (Table 3.7). Reduced disappearance, or increased appearance, of DM, N, and GE during Bhyo infection is expected due to hemorrhagic diarrhea, increased secretion of mucus, and cell sloughing (Argenzio et al., 1980; Albassam et al., 1985; Wilberts et al., 2014b). This could also be a reflection of altered microbial populations, and therefore microbial metabolites, commonly associated with Bhyo. Although microbial richness and diversity are reduced in pigs that develop clinical SD (Burrough et al., 2017), bacterial metabolism may be accelerated based on increased volatile fatty acid production (Siba et al., 1996). This increase in volatile fatty acid production and ATP in the hindgut correlated to increased clinical presentation of SD (Siba et al., 1996; Durmic et al., 2002). This likely contributes to the increased appearance of energy in the hindgut. Energy utilization by the hindgut can contribute to maintenance energy and improve feed efficiency (Dierick et al., 1990); however, an increased loss of energy in the hindgut of Bhyo pigs could suggest an increased energy need for the pigs.

Conclusion

Brachyspira hyodysenteriae, the classical agent of SD, antagonizes pig health and performance as a result of mucohemorrhagic diarrhea and colitis. In general, it is assumed that health challenges impact AID and ATTD; however, studies involving pathogenic agents that determine SID by correcting for BEL are limited. It is also unknown how different respiratory, systemic, or enteric pathogens and their disease progression modulates AA digestibility and requirements in pigs. This study aimed to determine how Bhyo modulates nutrient, energy, and

AA digestibility and BEL of AA. Contrary to what was hypothesized, these data suggest that Bhyo, which impacts the hindgut only, has minimal impact on BEL of AA and reduced SID of some AA. Therefore, Bhyo did not impact AA digestibility in the same way as other enteric pathogens and results are more similar to a systemic challenge. This suggests that not all diseases act similarly with regard to AA digestibility and metabolism and consideration should be given to pathogens individually. In addition, increased appearance of N and GE in the hindgut of Bhyo infected pigs is likely associated with decreased N and energy balance which likely attributes to reduced growth performance commonly seen with Bhyo infection. Therefore, we assume that the AA and energy needs are likely increased from decreased SID of N, Arg, Lys, and some nonessential AA, and a decreased energy contribution from the hindgut associated with Bhyo infection.

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Table 3.1. Diet composition, as-fed basis

Ingredient, %	Complete	NFD
Corn	75.56	-
Cornstarch	-	78.95
Soybean meal, 46.5% CP	19.86	-
Dextrose	-	10.00
Solka floc	-	4.00
Soybean oil	-	3.00
Casein	1.09	-
Monocalcium phosphate	0.82	1.35
Limestone	1.03	1.00
Salt	0.50	0.50
L-Lys·HCl	0.22	-
L-Thr	0.07	-
L-Trp	0.02	-
Chromic oxide	0.40	0.40
Potassium carbonate	-	0.40
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.15	0.15
Magnesium oxide	-	0.10

Complete = corn-soy diet; NFD = nitrogen-free diet

[1]Provided per kilogram of diet: 6,125 IU vitamin A, 700 IU vitamin D_3, 50 IU vitamin E, 30 mg vitamin K, 0.05 mg vitamin B_{12}, 11 mg riboflavin, 56 mg niacin, and 27 mg pantothenic acid.

[2]Provided per kilogram of diet: 22 mg Cu (as $CuSO_4$), 220 mg Fe (as $FeSO_4$), 0.4 mg I (as $Ca(IO_3)_2$), 52 mg Mn (as $MnSO_4$), 220 mg Zn (as $ZnSO_4$), and 0.4 mg Se (as Na_2SeO_3).

Table 3.2. Calculated and analyzed nutrient composition of experimental diets, as-fed basis

Parameter	Calculated		Analyzed	
	Complete	**NFD**	**Complete**	**NFD**
DM, %	88.6	-	94.8	96.3
Energy, Mcal/kg[1]	3.69	3.71	3.90	3.82
CP, %	16.7	0.20	15.6	0.73
Indispensable AA, %				
Arg	0.87	0.01	0.86	0.01
His	0.39	0.01	0.39	0.02
Ile	0.59	0.01	0.69	0.02
Leu	1.30	0.03	1.41	0.05
Lys	0.90	0.00	1.07	0.03
Met	0.24	0.00	0.31	0.01
Met + Cys	0.48	0.00	0.55	0.10
Phe	0.69	0.01	0.78	0.02
Thr	0.57	0.01	0.63	0.01
Trp	0.19	0.00	0.20	0.00
Val	0.66	0.01	0.76	0.02
Dispensable AA, %				
Ala	-	-	0.77	0.03
Asp	-	-	1.41	0.03
Cys	0.24	0.00	0.24	0.09
Glu	-	-	2.74	0.06
Gly	-	-	0.58	0.01
Pro	-	-	1.08	0.03
Ser	-	-	0.62	0.02
Tyr	0.47	0.01	0.53	0.01

Complete = corn-soy based diet; NFD = nitrogen-free diet.
[1]Calculated composition = Mcal ME/kg; analyzed composition = Mcal GE/kg.

Table 3.3. Pigs positive for and lesions frequency of *Brachyspira hyodysenteriae*

	Pre-inoculation	day post-inoculation					CC score frequency			
		5	7	9	12	14/15	0-2	3-5	6-9	P-value
Complete diet[2]										
Control (n=7 pigs)	0/7	0/7	0/7	0/7	0/7	0/7	7/7	0/7	0/7	0.258
Bhyo (n=10 pigs)	0/10	0/10	0/10	5/10	4/10	7/10	3/10	4/10	3/10	
NFD[3]										
Control (n=4 pigs)	0/4	-	-	0/4	0/4	0/4	4/4	0/4	0/4	
Bhyo (n=11 pigs)	0/11	-	-	5/11	4/11	1/11	8/11	3/11	0/11	

Bhyo = Brachyspira hyodysenteriae infected pigs; NFD = nitrogen-free diet.

[1]CC score = Composite colitis score reflecting the combined inflammatory scores from the cecum, spiral colon, and descending colon (max score = 9; detailed methods in the text).

[2]Positive by fecal culture for *Brachyspira hyodysenteriae.*

[3]PCR positive for *Brachyspira hyodysenteriae.*

Table 3.4. Apparent total tract and ileal digestibility coefficients in healthy and *Brachyspira hyodysenteriae* infected pigs

Parameter	Control	Bhyo	SEM	*P*-value
ATTD[1], %				
DM	88.16	85.16	0.79	0.017
N	84.60	78.13	1.73	0.019
GE	86.58	83.32	0.78	0.012
AID[2], %				
DM	86.86	86.55	0.71	0.759
N	85.98	85.74	0.60	0.788
Total AA	88.61	88.63	0.57	0.979
GE	86.83	86.24	0.66	0.541
Indispensable AA, %				
Arg	90.88	91.10	0.62	0.805
His	89.90	90.47	0.48	0.413
Ile	89.01	89.36	0.44	0.589
Leu	90.56	90.87	0.42	0.604
Lys	91.63	91.08	0.40	0.341
Met	92.93	92.75	0.37	0.734
Phe	89.54	89.89	0.43	0.576
Thr	86.32	86.51	0.49	0.796
Trp	88.98	89.90	0.42	0.146
Val	86.72	86.92	0.52	0.789
Dispensable AA, %				
Ala	87.12	86.88	0.63	0.793
Asp	88.05	88.69	0.40	0.278
Cys	83.50	84.51	0.65	0.289
Glu	91.67	92.16	0.50	0.506
Gly	76.78	80.14	1.04	0.039
Pro	86.97	87.92	1.08	0.543
Ser	87.67	87.82	0.44	0.806

Bhyo = *Brachyspira hyodysenteriae* infected pigs.
[1]ATTD = apparent total tract digestibility.
[2]AID = apparent ileal digestibility.

Table 3.5. Basal endogenous loss of AA (g/kg DMI) in healthy and *Brachyspira hyodysenteriae* infected pigs

Parameter	Control	Bhyo	SEM	*P*-value
Fecal N	1.03	4.89	2.84	0.355
Ileal N	4.40	2.71	0.70	0.113
Total AA	23.32	13.47	3.69	0.081
Indispensable AA				
Arg	1.31	0.69	0.24	0.092
His	0.21	0.17	0.03	0.361
Ile	0.31	0.27	0.04	0.562
Leu	0.52	0.45	0.07	0.499
Lys	0.47	0.40	0.06	0.393
Met	0.08	0.07	0.01	0.776
Phe	0.33	0.29	0.05	0.555
Thr	0.58	0.49	0.08	0.460
Trp	0.15	0.10	0.02	0.087
Val	0.51	0.43	0.08	0.482
Dispensable AA				
Ala	0.82	0.53	0.14	0.165
Asp	0.87	0.69	0.13	0.325
Cys	0.23	0.18	0.03	0.286
Glu	0.98	0.78	0.13	0.294
Gly	3.04	1.72	0.52	0.096
Pro	11.95	5.38	2.11	0.046
Ser	0.60	0.45	0.09	0.238
Tyr	0.29	0.25	0.04	0.486

Bhyo = *Brachyspira hyodysenteriae* infected pigs.

Table 3.6. Standardized ileal digestibility coefficients in healthy and *Brachyspira hyodysenteriae* infected pigs

Parameter	Control	Bhyo	SEM	*P*-value
N	103.59	96.49	0.60	<0.001
Total AA	103.21	97.06	0.57	<0.001
Indispensable AA, %				
Arg	105.27	98.72	0.62	<0.001
His	95.07	94.56	0.48	0.464
Ile	93.27	93.12	0.44	0.805
Leu	94.05	93.87	0.42	0.764
Lys	95.79	94.58	0.40	0.047
Met	95.23	94.92	0.37	0.565
Phe	93.55	93.43	0.43	0.836
Thr	94.98	93.41	0.61	0.088
Trp	95.33	94.77	0.50	0.439
Val	93.05	92.27	0.52	0.300
Dispensable AA, %				
Ala	97.25	93.40	0.63	0.001
Asp	93.89	92.90	0.51	0.189
Cys	92.39	91.66	0.65	0.440
Glu	95.07	94.85	0.50	0.753
Gly	126.51	106.47	1.77	<0.001
Pro	191.85	131.03	3.63	<0.001
Ser	96.84	94.71	0.44	0.004
Tyr	94.30	93.89	0.40	0.475

Bhyo = *Brachyspira hyodysenteriae* infected pigs.

Table 3.7. Hindgut nutrient and energy disappearance in healthy and *Brachyspira hyodysenteriae* infected pigs fed complete or nitrogen-free diet

	Complete				NFD			
Parameter	**Control**	**Bhyo**	**SEM**	***P*-value**	**Control**	**Bhyo**	**SEM**	***P*-value**
DM, g/d	21.44	-21.04	14.36	0.055	127.49	0.25	70.84	0.226
N, g/d	-0.05	-2.93	0.74	0.036	5.87	-4.28	5.50	0.215
GE, Mcal/d	-20.2	-188.2	50.65	0.033	462.5	-289.4	456.13	0.265

Positive value denotes disappearance, negative value denotes appearance
Bhyo = *Brachyspira hyodysenteriae* infected pigs;
Complete = corn-soy based diet; NFD = nitrogen-free diet

CHAPTER 4. INCREASED LYSINE:ME RATIO IMPROVES GROWER PIG PERFORMANCE DURING A PRRSV CHALLENGE

A manuscript prepared for the *Journal of Animal Science*

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) significantly reduces pig performance. The AA requirements and Lys:ME of health-challenged pigs for optimum performance are poorly understood. Two experiments were conducted to evaluate the effect of SID Lys:ME (g SID Lys per Mcal ME) on growth performance during a PRRSV challenge. In Exp. 1, 379 barrows (51.3 ± 0.3 kg BW) were allotted to one of six diets (1.87 to 3.41 Lys:ME) for a 35 d growth study. In Exp. 2, 389 barrows (29.2 ± 0.23 kg BW) were allotted to one of six diets (2.39 to 3.91 Lys:ME) for a 49 d growth study. These isocaloric diets represented 80 to 130% of NRC SID Lys requirement. For each Exp., pigs were randomly allocated across two barns of 24 pens each with 7-9 pigs/pen (4 pens/diet/health status). On day 0, one barn was

inoculated with live PRRSV, one barn sham inoculated (control), and all pigs were started on experimental diets. Pen growth performance and feed intake were recorded weekly and G:F calculated. Breakpoint analysis was used to determine the Lys:ME ratio that maximized ADG and G:F over the 35 or 49 d test periods for Exp. 1 and 2, respectively. In Exp. 1 increasing Lys:ME increased ADG (quadratic $P = 0.01$) and G:F (linear and quadratic $P = 0.04$) in control pigs over 35 d. In PRRSV pigs, ADG and G:F increased linearly with increasing Lys:ME ($P < 0.01$). The Lys:ME for optimum ADG and G:F during PRRSV challenge was 2.83 and 3.17, respectively, compared to 2.24 and 2.83, respectively, in control pigs using a one-slope broken-line model. In Exp. 2, pigs in the control barn became naturally infected after 21 dpi. Prior to infection, ADG and G:F increased with increasing Lys:ME in control and PRRSV pigs (linear and quadratic $P < 0.05$), and optimum ADG and G:F were achieved at 3.02 and 2.92 Lys:ME, respectively, in PRRSV pigs compared to 2.82 and 3.22 Lys:ME, respectively, in control pigs. Over the 49 d period, increasing Lys:ME improved ADG ($P < 0.01$, linear and quadratic) and G:F (linear $P < 0.01$) in naturally infected pigs. The response was similar in experimental infection for ADG ($P < 0.01$, linear and quadratic) and G:F (linear $P = 0.01$). The optimum ratio for ADG (2.86 vs. 3.12 Lys:ME) and G:F (3.18 vs. 3.08 Lys:ME) was similar between natural and experimental infection. In summary, increasing Lys:ME ratio by 110 to 120% improved performance and feed efficiency during a PRRSV challenge. This response was similar in experimental and natural PRRSV infections.

KEYWORDS: pig, PRRSV, performance, Lys:ME, breakpoint analysis

Introduction

Nutritional requirements have been well established for healthy pigs; however, requirements for pigs facing health challenges are largely unexplored, particularly AA requirements and these requirements in relation to energy intakes. It has been established that pig performance and lean tissue accretion rates are decreased due to different pathogens (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017; Helm et al., 2018); however, it is not known if this is a result of decreased feed intake. Additionally, this may be due to a repartitioning of nutrients, specifically AA, to meet altered metabolic and immune needs (Klasing and Calvert 1999). Lysine is the first limiting AA for healthy pigs fed corn-soybean meal diets; however, AA pertinent to the immune system and its activation may differ from that of growth (Reeds et al., 1994; Le Floc'h et al., 2004).

Interestingly, Lys requirements (g/d basis) are reduced in immune-stimulated pigs compared to control pigs (Williams et al., 1997b, c). This is due to a greater capacity for proteinaceous tissue accretion in healthy pigs as partial efficiency for Lys utilization may not be altered due to health status (Williams et al., 1997a). In addition, adequate energy is essential for a proper immune response. Diets deficient in protein and energy can lead to reduced growth during parasite infection (Pedersen et al., 2002).

Porcine reproductive and respiratory syndrome virus (**PRRSV**) is one of the most economically significant pathogens to the swine industry. However, research pertaining to this virus's impact on nutritional requirements in pigs is minimal. Our group has recently reported in growing pigs that PRRSV reduces lean tissue accretion rates (Schweer et al., 2017), but basal endogenous losses of many AA and standardized ileal digestibilities (**SID**) of AA are not different (Chapter 2). Therefore, the objective of these studies was to evaluate the effects of

graded levels of g SID Lys per Mcal ME (**Lys:ME**) on pig performance during a health challenge in the growing phase. This will allow for the optimal Lys:ME to be defined for PRRSV challenged pigs.

Materials and Methods

All procedures adhered to the ethical and humane use of animals for research and were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 8-16-8330-S).

Two experiments were conducted to determine the ideal SID Lys:ME ratio for grow-finish barrows (purebred Maschhoffs proprietary line Duroc sires by commercial Yorkshire-Landrace F1 females) during a PRRSV challenge. In both experiments, pigs were split across two identical barns of which one was maintained as a PRRS negative control and the other inoculated with a live strain of PRRSV (open reading frame 5 sequence 1-18-4). Pigs in the PRRSV barn were inoculated on days post inoculation (**dpi**) 0 with 2 mL of live PRRSV (1 mL intramuscular and 1 mL intranasal; 10^6 genomic PRRSV units per mL) while the control barn received a sham saline inoculation. Pigs were allowed unrestricted access to feed and water. During the challenge period, pigs were fed one of six experimental diets; body weight and feed disappearance were measured weekly to determine ADG, ADFI, and to calculate G:F. Experimental diets were corn-soybean meal based and were formulated to be isocaloric and meet or exceed the nutritional requirements of 50-100 kg and 25-50 kg pigs in Exp. 1 and 2, respectively (NRC, 2012). There was a stepwise increase in SID Lys:ME ratio and ratios of SID Thr, Trp, Met, Ile, and Val to SID Lys were held constant. The dietary SID Lys:ME levels were achieved by increasing soybean meal. By design, as Lys:ME increased so did CP, but the

essential AA to Lys ratios were maintained using crystalline AA. The diet was formulated so a majority of the SID Lys requirement was met with soybean meal to maintain commercial relevance. These diets correlated to 80, 90, 100, 110, 120, and 130% of NRC (2012) Lys requirement as verified internally for in the Maschhoffs system for 50-100 kg and 25-50 kg pigs used in Exp. 1 and 2, respectively.

Experiment 1, 50-100 kg BW pigs

In Exp. 1, 379 barrows (51.3 ± 0.32 kg BW) were randomly allotted to one of six dietary treatments with 4 pens per treatment per health status and 7-8 pigs per pen. Prior to arrival, all pigs were vaccinated for *Mycoplasma hyopneumoniae*, porcine circovirus, erysipelas, and ileitis, and were serologically negative for PRRS virus as determined by PCR. Pigs were given a 14 d acclimation period during which all pigs were fed a common diet. At the time of PRRSV inoculation, pigs were started on experimental diets (Table 4.1), and performance was measured for 35 d. Diets were formulated to contain 1.87, 2.18, 2.49, 2.80, 3.11, and 3.41 SID Lys:ME ratio, representing 80, 90, 100, 110, 120, and 130% of NRC requirement, respectively. Weekly after PRRSV inoculation, the same two pigs per pen were bled for PRRSV PCR and ELISA. After the experimental period, all pigs were fed a common multi-phase diet until pigs reached market BW (approximately 128 kg BW), at which time pigs were slaughtered, and carcass data collected from the slaughter plant (JBS, Marshalltown, IA). Shipping and pre-slaughter handling were the same for control and PRRSV pigs.

Experiment 2, 25-50 kg BW pigs

In Exp. 2, 389 barrows (29.2 ± 0.23 kg BW) were vaccinated for *Mycoplasma hyopneumoniae*, porcine circovirus, erysipelas, and ileitis prior to arrival, and serologically negative for PRRS virus as determined by PCR. Barrows were randomly allotted to one of six

dietary treatments formulated to contain 2.33, 2.63, 2.94, 3.24, 3.55, and 3.85 SID Lys:ME, representing 80, 90, 100, 110, 120, and 130% of NRC requirement, respectively (Table 4.2). Each treatment had 4 pens per treatment per health status with 7-9 pigs per pen. After a 10 d acclimation on a common diet, pigs were inoculated with PRRSV and started on experimental diets for a 49 d growth study. Between 21 and 28 dpi, the control barn became naturally infected with PRRSV and were confirmed positive by serum PCR. The PRRSV strain isolated from the control barn was considered identical to the challenge isolate used in the PRRS barn by ORF-5 sequence. In Exp. 2, carcass data was unable to be obtained from the slaughter plant.

Diet Analysis

Proximate analysis of diets in both experiments were carried out in a commercial laboratory (Midwest Labs, Omaha, NE). Dietary AA and N analysis were conducted by Ajinomoto Heartland, Inc., (Eddyville, IA). Amino acid and N analysis were performed using method 994.12, 999.13, and 990.03 according to AOAC (2007) methods, and CP was calculated (N × 6.25).

Blood Collection and Analysis

In both experiments, 8 to 10 mL blood samples were collected from the jugular vein into serum tubes (BD Vacutainer, Franklin Lakes, NJ) while pigs were snare restrained. In Exp. 1, the same two pigs per pen were bled weekly during the 35 d challenge period. In Exp. 2, 6 pigs/room (12 pigs/barn) were randomly selected and bled weekly during the 49 d challenge period. Serum from these pigs was pooled within room after centrifugation. Serum was allowed to clot then separated by centrifugation (2,000 × *g*, 15 min at 4°C), aliquoted and submitted to the Iowa State University Veterinary Diagnostic Laboratory. Real-time RT-PCR and serum antibody testing for PRRSV was performed using commercial reagents (VetMAX™ NA and EU PRRSV real-time

RT-PCR, Thermo Fisher Scientific, Waltham, MA) and a commercial ELISA kit (HerdCheck® PRRS X3, IDEXX Laboratories, Inc., Westbrook, ME), respectively.

Statistics

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Car, NC) for linear and quadratic effects of increasing SID Lys:ME. Pen served as the experimental unit in both experiments. Data were considered significant if $P \leq 0.05$ and a trend if $P \leq 0.10$. For both experiments, one-slope straight broken-line and quadratic broken-line analysis as described by Robbins et al. (2006) were used to estimate SID Lys:ME requirement for ADG and G:F of the treatments (control or PRRS pigs). Breakpoint analysis was determined separately for control and PRRSV infected pigs and compared. In Exp. 1, breakpoint analysis was performed on performance over the 35 d experimental period. In Exp. 2, breakpoint analysis was performed on performance from 0-21 dpi, the period for which control pigs were negative for PRRSV. In Exp. 2, breakpoint analysis was also performed over the 49 d experimental period to determine if SID Lys:ME requirements are similar between pigs naturally and experimentally infected with PRRSV.

Results

Diet Analysis

Experimental diets were formulated to contain 1.87, 2.18, 2.49, 2.80, 3.11, and 3.41 and 2.33, 2.63, 2.94, 3.24, 3.55, and 3.85 g SID Lys per Mcal ME in Exp. 1 and 2, respectively based on analyzed Lys and GE, and predicted ME values (Table 4.1 and Table 4.2, respectively). Proximate and AA analysis of the diets determined that experimental diets were formulated similarly to the predicted or calculated values. The ratio of SID Thr, Met+Cys, Trp, Ile, and Val

to SID Lys remained constant across all diets. As expected, dietary energy was not different, and CP increased as soybean meal inclusion increased.

Experiment 1

In Exp. 1, there were two mortalities in the control barn and three in the PRRS barn. Both pigs in the control barn succumbed to hemorrhagic bowel syndrome. Two unthrifty pigs in the PRRS barn were euthanized shortly after arrival, and the third was determined to expire from porcine dermatitis and nephropathy syndrome. There were no associations between mortality and dietary treatment.

Prior to experimental inoculation with PRRSV in both experiments, all pigs were negative for PRRS virus and antibody. In Exp. 1, control pigs remained negative for PRRS virus and antibody throughout the 35 d experimental diet period and to market, as expected. No diet or diet × dpi interactions were detected for PRRSV PCR Ct value or Log_{10} PRRSV genomic content (Table 4.3). Similarly, no differences were detected for PRRSV antibody ($P > 0.10$). Expectedly, PRRSV Ct value and Log_{10} genomic content decreased over time while PRRSV antibody increased causing a main effect of dpi ($P < 0.001$).

Prior to feeding experimental diets and inoculation, growth performance and feed efficiency were not different in control or PRRSV pigs (Table 4.4). Over the 35 d test period, ADG and G:F in control pigs increased as SID Lys:ME increased (quadratic, $P < 0.05$ both parameters). Feed intake was not different over the 35 d test period in control pigs. In the post-challenge period, when all pigs were on a common diet, there were no performance differences ($P > 0.10$, data not shown). Pig growth and feed intake from 0 dpi to market (76 d period) were not different ($P > 0.10$); however, G:F increased up to 3.11 SID Lys:ME resulting in a significant quadratic effect ($P = 0.040$). Over the 35 d period, ADG and G:F of PRRSV pigs increased

linearly with increasing SID Lys:ME (P = 0.001 and P = 0.002, respectively), and ADFI tended to increase (linear, P = 0.068). Similar to control pigs, there was no difference after 35 dpi when all pigs were on a common diet (data not shown). From inoculation to market (78 ± 2 d), ADG increased linearly with increasing SID Lys:ME (P = 0.011); however, ADFI and G:F were not different (P > 0.10).

Breakpoint analysis was used to determine the optimal SID Lys:ME ratio to maximize growth and feed efficiency in control and PRRSV pigs (Fig. 4.1). It was determined that optimal ADG in non-infected control pigs was achieved at 2.24 and 2.38 SID Lys:ME using a one-slope and quadratic broken-line model, respectively. Optimal G:F was achieved at 2.83 and 2.95 Lys:ME in a one-slope and quadratic broken-line model, respectively. In PRRSV infected pigs, optimal ADG and G:F were achieved at 2.83 and 3.17 SID Lys:ME, respectively, using a one-slope broken-line model. When using a quadratic broken-line model the optimal ADG and G:F were predicted to be 4.71 and 4.22 SID Lys:ME, respectively; however, these values are outside of the maximum SID 3.41 Lys:ME diet tested and should be studied further.

Carcass composition was evaluated when pigs reached approximately 128 kg BW (Table 4.5). All control pigs were marketed at 76 dpi, and there was no difference in final BW (P > 0.10). There was a quadratic effect (P = 0.016) of SID Lys:ME on fat depth where fat depth decreased from 1.87 to 2.80 SID Lys:ME and increased from 2.80 to 3.41 SID Lys:ME. Concurrently, there was a linear tendency (P = 0.060) for lean percentage to increase as SID Lys:ME increased. Hot carcass weight (**HCW**) and dress percentage were not impacted by increasing SID Lys:ME in control pigs. In PRRSV infected pigs, fat depth increased linearly (P = 0.045), and lean depth showed a strong tendency (P = 0.059) to decrease with increasing SID

Lys:ME. Days to market decreased from 80 to 77 days as SID Lys:ME increased (linear, P = 0.004).

Experiment 2

The control and PRRS barn experienced three and nine mortalities, respectively. Two mortalities in the control barn were a result of porcine dermatitis and nephropathy syndrome and one from hemorrhagic bowel syndrome. In the PRRS barn, five mortalities were a result of secondary respiratory infection, two due to gastric ulcers, one to rectal prolapse, and one to bacterial endocarditis. In both barns, there were no treatment effects on mortality. Pigs responded more severely to PRRSV infection than anticipated, therefore to decrease the impact of opportunist bacteria and avoid a high number of mortalities, antibiotics were delivered through the water for the entire barn from 11-14 dpi.

As there were no differences in PRRS viremia or antibody attributed to diet in Exp. 1, pigs in Exp. 2 were randomly bled across diets to confirm PRRSV infection status in control pigs (Table 4.6). In Exp. 2, control pigs remained PRRSV negative until 21 dpi; however, after 21 dpi, the control pigs were naturally infected with the same PRRSV isolate used for experimental infection (open reading frame 5 sequence 1-18-4). The control pigs became infected with PRRSV around 21 dpi, therefore, data were analyzed as two separate challenge periods. The first challenge period, 0-21 dpi, represents when control pigs were not infected with PRRSV. The second period, 0-49 dpi, is to determine the impact of a natural PRRSV infection compared to experimental infection.

Prior to experimental infection at 0 dpi, control pig performance and feed efficiency were not different (Table 4.7). During the first challenge period (0-21 dpi) when control pigs were uninfected, ADG (linear $P < 0.001$, quadratic $P = 0.020$) and G:F (linear $P < 0.001$) increased as

Lys:ME increased. Feed intake increased from 2.33 to 3.24 Lys:ME and then decreased, resulting in a quadratic effect ($P = 0.039$). When breakpoint analysis was performed on 0-21 dpi performance, optimal ADG and G:F was achieved at 2.82 and 3.22 Lys:ME, respectively, in a one-slope broken-line model. In a quadratic broken-line model, optimal ADG was attained at 3.32 Lys:ME. Optimal G:F was predicted at 4.22 Lys:ME; however, this was outside the range of the experimental diets tested. Although PRRSV pigs were on a common diet prior to experimental infection, ADG and G:F increased linearly ($P < 0.01$); however, differences in ADG and G:F prior to infection did not significantly impact performance in other experimental periods. In PRRSV pigs, 21 d ADG, ADFI, and G:F increased linearly with increasing Lys:ME ($P \leq 0.001$, all parameters), and ADG and G:F also demonstrated a quadratic effect ($P = 0.043$ and $P = 0.006$, respectively). Breakpoint analysis determined optimal ADG and G:F at 3.02 and 2.92, respectively, in a one-slope broken-line model and 3.41 and 3.22, respectively, in a quadratic broken-line model.

In Exp. 2, control pigs became infected with PRRSV after 21 dpi. Therefore, performance and feed efficiency were evaluated from 0-49 dpi to determine the effect of natural versus experimental infection. In pigs naturally infected with PRRSV, ADG increased linearly from 2.33 to 3.24 with increasing Lys:ME resulting in both linear and quadratic effects ($P < 0.001$ and $P = 0.003$, respectively). Also, in naturally infected pigs, ADFI increased quadratically ($P = 0.029$) with a peak at 3.24 Lys:ME and G:F increased linearly ($P < 0.001$) and with Lys:ME. From 0 dpi to market (approximately 100 d), ADG increased as Lys:ME increased, causing an increase in final BW (linear $P < 0.02$, quadratic $P < 0.01$, both parameters). Overall feed intake increased in a quadratic manner ($P = 0.032$). Breakpoint analysis determined 2.85 and 3.41 Lys:ME for optimal ADG using one-slope and quadratic broken-line models,

respectively (Fig. 4.2). Optimal G:F Lys:ME was achieved at 3.18 and 3.85 in one-slope and quadratic broken-line models, respectively.

Pigs experimentally infected with PRRSV demonstrated a similar response to increasing Lys:ME, with ADG and ADFI having a linear ($P < 0.001$, both parameters) and quadratic ($P = 0.007$ and $P = 0.037$, respectively) response, while G:F responded linearly to increasing Lys:ME ($P = 0.011$). Overall, final BW and ADG increased linearly with Lys:ME (linear $P \leq 0.002$, both parameters). Feed intake increased from 2.33 to 3.24 Lys:ME then decreased, leading to a linear ($P < 0.001$) and quadratic effect ($P = 0.048$). Optimal ADG was achieved at 3.12 and 3.47 Lys:ME using one-slope and quadratic broken-line breakpoint analysis, respectively (Fig. 4.3). Optimal G:F was achieved at 3.08 and 3.52 Lys:ME using one-slope and quadratic broken-line models, respectively.

Discussion

In healthy growing pigs, Lys is the first limiting AA for growth, and recommendations for Lys requirements have been widely established (NRC, 2012). Interestingly, when pigs are housed in unsanitary conditions, the Lys requirement for growth is reduced (Williams et al., 1997b, c), which has been attributed to a reduced capacity for protein accretion (Williams et al., 1997a); however, efficiency of Lys utilization may not be different between healthy and immune-stimulated pigs. Therefore, reduced feed intake, and thus Lys intake, likely contributes to the reduction in lean tissue accretion and growth. In a similar unsanitary model, van der Meer et al. (2016) reported an improvement in feed efficiency when Met, Thr, and Trp were increased 20% relative to Lys. In contrast, when immune system activation was modeled using repeated LPS, Met+Cys requirement was reduced (Rakhshandeh et al., 2014), but Met:Met+Cys requirement for protein deposition increased (Litvak et al., 2013). These data suggest that AA

requirements in a model that mimics inflammation may be different from that of healthy pigs. Because pigs eat to meet their energy requirement and feed intake is typically reduced during a health challenge, nutrient requirements may be better expressed as their relationship to energy content in the diet. Therefore, we conducted two experiments to determine how increasing Lys:ME impacted growth performance in healthy and PRRSV challenge pigs.

To our knowledge, this is the first set of experiments to determine the Lys:ME requirements for optimal ADG and G:F in pigs challenged with PRRSV. Compared to healthy cohorts in Exp. 1, PRRSV increased Lys:ME requirement for ADG by 21 to 36% depending on the statistical model used; however, the quadratic model predicted Lys:ME requirement to be 3.71 Lys:ME which is above the 3.41 Lys:ME tested in the study suggesting that the requirement could be higher than the test diets. Similarly, PRRSV increased the Lys:ME for optimal G:F by 11% to 30%. Similar to the predicted quadratic requirement for ADG, the G:F prediction was above the 3.41 Lys:ME diet and, therefore, the requirement may be higher than the tested diets. In Exp. 2, and in agreement with Exp. 1, optimal ADG was achieved at 3% to 7% higher Lys:ME in PRRSV pigs compared to healthy controls. Interestingly, PRRSV decreased Lys:ME requirement to achieve optimal G:F by 9 to 25%; however, optimal G:F in control pigs using a quadratic model predicted a requirement above the diets tested. Because control pigs in Exp. 2 became infected with PRRSV, the optimal Lys:ME was able to be determined for natural versus experimental PRRSV infection. Interestingly, optimal ADG and G:F was achieved at slightly higher Lys:ME levels in naturally infected pigs compared to experimentally infected cohorts. These data contrast with the classic papers by Williams et al. (1997a, b, c) that determined Lys requirements to be less in immune-stimulated pigs compared to healthy pigs; however, Lys efficiency was not different between groups suggesting growth differences are related to feed

intake and Lys intake. A similar response occurs in broilers challenged with LPS, where Lys utilization by muscle does not change, but Lys utilization by the immune system increases 6-fold (Klasing and Calvert, 1999). As mentioned, soybean meal was used to increase dietary Lys, therefore, intake of other AA are likely increased. Acute-phase protein synthesis requires a large portion of aromatic AA (Reeds et al., 1994). Also, increased Met and Met+Cys can be beneficial to protein deposition in LPS challenged pigs (Litvak et al., 2013; Rakhshandeh et al., 2014). Altogether, increased intake of these AA and others can reduce the need for lean tissue catabolism and preserve lean tissue and therefore growth.

In Exp. 1, although ADG was different between control and PRRSV pigs (1.14 vs. 0.86 kg/d, respectively), growth was optimized at similar total Lys intake of 22 and 21.5 g/d for control and PRRSV pigs, respectively, and both were similar to the recommended 20.5 g/d total Lys intake for 50-75 kg pigs by the NRC (2012). Although growth was different, it was optimized at similar Lys intake which is somewhat similar to results from Williams et al. (1997b, 1997c) where growth was similar at similar Lys intake; however, this study utilized an unsanitary environment challenge model, not a live virus. In Exp. 2, 0-21 dpi ADG in control and PRRSV pigs was maximized at 18.7 and 10.6 g/d total Lys intake, respectively. Control pigs were similar to the 16.9 g/d total Lys intake recommended by the NRC for 25-50 kg pigs. Although PRRSV pigs were well below NRC recommendation total Lys intake was similar to the 12.8 g/d Lys intake estimated by Williams et al. (1997c) for optimal growth in 25 kg pigs immune-stimulated by an unsanitary environment. Infection appeared more severe in Exp. 2 as compared to Exp. 1, likely because pigs were younger, therefore, more severe infection could result in Lys efficiency differences.

When pigs are experimentally infected with a pathogen, the population is on the same disease plane as opposed to a natural infection that can lead to persistent, recurring infection (Yoon et al., 1999; Chand et al., 2012). Pigs that experienced natural and experimental PRRSV infection reached a similar peak viremia based on Ct values and similar peak antibody. Naturally infected pigs appear to have experienced a shorter viremia duration; however, the same pigs were not bled for the duration of the growth period to more accurately determine PRRS virus and antibody dynamics. Pigs became naturally infected around 45 kg which likely allowed them to cope better with disease. As mentioned, pigs naturally and experimentally infected with PRRSV had a similar Lys:ME for optimal growth. Therefore, it is difficult to elucidate the effect of BW or diet on a potential protective role of against chronic PRRSV infection.

In the U.S. swine industry, soybean meal a key feedstuff used to increase Lys and essential AA concentrations in the diet. Feeding increased soybean meal levels to PRRSV infected pigs may also have potential benefits for PRRS viral clearance (Rochell et al., 2015); however, Lys:ME was not different between diets. In the study presented herein, altering Lys:ME by increasing soybean meal content of the diets did not alter viral titers, PRRSV genomic content or antibody response within the PRRSV challenged pigs (Exp. 1). Although contrary to Rochell et al., (2015), this result is consistent with a previous study from our group (Chapter 2). Increased dietary soybean meal, regardless of Lys:ME ratio can also increase performance in finishing pigs naturally infected with PRRSV and porcine circoviral disease (Boyd et al., 2010). In 8 kg pigs infected with PRRSV, ADG was also improved in a soybean meal diet versus a soybean meal plus crystalline AA diet with the same Lys:ME (Rochell et al., 2015). A similar, linear increase in ADG and G:F was seen in 55 kg pigs that were PRRSV positive at weaning (Shelton et al., 2011). In agreement with these reports, pigs infected with

PRRSV in the current study showed linear improvements in ADG and G:F as Lys:ME increased suggesting a potential role of soybean meal. In contrast, previous data from our group suggests no benefit of increased soybean meal in late-finishing pigs dual-challenged with *Mycoplasma hyopneumoniae* and PRRSV (O'Connell et al., 2016). These potential roles for soybean meal to improve growth during different health challenges are likely a result of increased nonessential AA or CP, as Lys:ME was not different in many studies.

In agreement with Li et al. (2012), fat thickness was impacted by Lys:ME in control pigs. In the current study there was a clear quadratic effect while Li et al. demonstrated both a linear and quadratic effect; however, in the current study experimental diets were not fed up until carcass data was collected as was the case in the study performed by Li et al. (2012). Interestingly, when comparing fat thickness in control and PRRSV pigs, there is an opposite effect of Lys:ME, where fat thickness decreased from 1.87 to 2.80 Lys:ME in control pigs and then increased up to 3.41 Lys:ME. In PRRSV pigs, fat depth increased linearly with increasing Lys:ME. Our group has shown that fat accretion is decreased in PRRSV infected pigs (Schweer et al., 2017), therefore, increasing the AA profile of the diet during a PRRSV challenge period may aid in maintaining energy levels and therefore body fat. In Exp. 1, although growth rates were different during the challenge period control and PRRSV infected pigs reached finishing weight at the same time suggesting the possibility of compensatory growth. Compensatory growth is a phenomenon where pigs accelerate growth after a period of feed or nutrient restriction, although this is not consistently observed (Mersmann et al., 1987; Taylor et al., 2013).

In summary, increased Lys:ME during a 35 d or 21 d PRRSV challenge in 50 and 25 kg pigs, respectively, increases ADG and G:F. There was no difference in immune response, as

determined by PRRS viremia or antibody response, and no difference in carcass characteristics. When breakpoint analysis was performed in Exp. 1, optimal Lys:ME for ADG and G:F was increased up to 36% and 30%, respectively, in PRRSV infected pigs compared to healthy controls. In Exp. 2, optimal Lys:ME for ADG increased up to 7%; however, optimal Lys:ME for G:F was decreased up to 25% in PRRSV infected pigs. In Exp. 1, the predicted requirement for ADG and G:F in PRRSV pigs using a quadratic model were above the highest Lys:ME diet. This was similar for G:F in control pigs in Exp. 2, therefore, further studies should be conducted to more accurately determine the Lys:ME requirement. In Exp. 2, it was also determined that Lys:ME for optimal ADG and G:F between pigs naturally and experimentally infected with PRRSV was not different. Altogether, increasing Lys:ME above the NRC requirement increased performance and feed efficiency in PRRSV infected pigs, and the response was similar between natural and experimental PRRSV infection.

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Table 4.1. Experiment 1 diet composition, as fed basis

	SID[1] Lys:ME (g/Mcal)					
Ingredients, %	1.87	2.18	2.49	2.80	3.11	3.41
Corn	87.16	84.13	81.07	77.74	73.95	70.29
Soybean meal, 48%	9.75	12.75	15.74	19.10	22.92	26.61
Limestone	1.00	1.01	1.02	1.02	1.02	1.03
Monocalcium phosphate, 21%	1.13	1.07	1.05	0.94	0.86	0.79
Salt	0.51	0.51	0.51	0.51	0.51	0.51
L-Lysine·HCl	0.27	0.31	0.34	0.37	0.38	0.39
Commercial VTM[2]	0.11	0.11	0.11	0.11	0.11	0.11
L-Threonine	0.05	0.06	0.08	0.10	0.10	0.11
DL-Methionine	0.02	0.05	0.08	0.11	0.13	0.15
Optiphos 1000	-	-	-	0.004	0.006	0.009
Calculated composition						
DM, %	85.6	85.7	85.8	85.9	86.0	86.1
CP, %	11.4	12.6	13.9	15.2	16.8	18.3
ME, Mcal/kg	3.28	3.27	3.27	3.27	3.27	3.26
SID AA						
Lys	0.61	0.71	0.81	0.92	1.02	1.11
Thr:Lys	0.60	0.60	0.60	0.60	0.60	0.60
Met:Lys	0.30	0.32	0.33	0.34	0.35	0.35
Met+Cys:Lys	0.57	0.57	0.57	0.57	0.57	0.57
Trp:Lys	0.16	0.16	0.16	0.16	0.17	0.17
Ile:Lys	0.58	0.58	0.57	0.57	0.58	0.59
Val:Lys	0.71	0.68	0.66	0.65	0.65	0.65
SID Lys:ME, g/Mcal	1.87	2.18	2.49	2.80	3.11	3.41
Total Lys, %	0.70	0.80	0.91	1.02	1.13	1.23
Analyzed composition						
DM, %	85.8	85.8	85.9	87.3	87.1	87.0
CP, %	13.6	15.3	16.3	18.4	20.3	22.8
Thr:Lys	0.61	0.63	0.61	0.62	0.67	0.59
Met:Lys	0.28	0.29	0.29	0.31	0.33	0.28
Met+Cys:Lys	0.58	0.58	0.55	0.57	0.59	0.50
Trp:Lys	0.15	0.16	0.16	0.16	0.19	0.17
Ile:Lys	0.57	0.56	0.64	0.65	0.68	0.56
Val:Lys	0.71	0.68	0.72	0.72	0.74	0.63
Total Lys, %	0.76	0.87	0.96	1.04	1.14	1.27

[1]SID = standardized ileal digestible.

[2]VTM=Vitamin-trace mineral premix, which supplied per kilogram of diet: vitamin A, 8,820 IU; vitamin D_3, 1,653 IU; vitamin E, 33.1 IU; vitamin K, 4.4 mg; riboflavin, 6.6 mg; niacin, 38.9 mg; pantothenic acid, 22.1 mg; vitamin B_{12}, 0.04 mg; I, 1.1 mg as potassium iodide; Se, 0.30 mg sodium selenite; Zn, 60.6 mg as zinc oxide; Fe, 36.4 mg as ferrous sulfate; Mn, 12.1 mg as manganous oxide; and Cu, 3.6 mg as copper sulfate.

Table 4.2. Experiment 2 diet composition, as fed basis

Ingredients, %	**SID Lys:ME (g/Mcal)**					
	2.33	2.63	2.94	3.24	3.55	3.85
Corn	82.16	79.11	75.59	71.87	68.02	64.29
Soybean meal, 48% CP	14.55	17.52	21.08	24.84	28.73	32.49
Limestone	0.96	0.98	0.98	0.99	0.99	1.00
Monocalcium phosphate, 21%	1.01	0.99	0.89	0.80	0.72	0.64
Salt	0.51	0.51	0.51	0.51	0.51	0.51
L-Lysine·HCl	0.32	0.35	0.37	0.38	0.39	0.40
Beef tallow	0.25	0.25	0.25	0.25	0.25	0.25
Commercial VTM[1]	0.11	0.11	0.11	0.11	0.11	0.11
L-Threonine	0.07	0.09	0.10	0.10	0.11	0.12
DL-Methionine	0.06	0.09	0.12	0.14	0.16	0.18
Optiphos 1000	0.0	0.0	0.008	0.008	0.009	0.012
Calculated composition						
DM, %	86.2	86.3	86.3	86.4	86.5	86.6
CP, %	13.0	14.2	15.6	17.2	18.7	20.2
ME, Mcal/kg	3.29	3.29	3.29	3.29	3.29	3.29
SID AA						
Lys	0.77	0.86	0.97	1.07	1.17	1.27
Thr:Lys	0.60	0.60	0.60	0.60	0.60	0.60
Met:Lys	0.32	0.33	0.34	0.34	0.35	0.35
Met+Cys:Lys	0.57	0.57	0.57	0.57	0.57	0.57
Trp:Lys	0.16	0.16	0.16	0.17	0.17	0.17
Ile:Lys	0.58	0.58	0.58	0.59	0.59	0.60
Val:Lys	0.67	0.66	0.65	0.65	0.65	0.65
SID Lys:ME, g/Mcal	2.33	2.63	2.94	3.24	3.55	3.85
Lys, Total %	0.86	0.97	1.08	1.18	1.29	1.40
Analyzed composition						
DM, %	86.3	86.1	86.5	86.6	86.5	86.8
CP, %	14.1	15.7	16.1	17.9	20.2	20.8
Thr:Lys	0.57	0.61	0.64	0.61	0.62	0.61
Met:Lys	0.26	0.31	0.32	0.32	0.30	0.30
Met+Cys:Lys	0.49	0.57	0.56	0.55	0.53	0.53
Trp:Lys	0.15	0.18	0.18	0.17	0.18	0.17
Ile:Lys	0.53	0.64	0.64	0.59	0.61	0.62
Val:Lys	0.63	0.71	0.70	0.64	0.66	0.66
Lys, Total %	1.00	1.00	1.07	1.21	1.38	1.44

[1]SID = standardized ileal digestible.

[2]VTM=Vitamin-trace mineral premix, which supplied per kilogram of diet: vitamin A, 8,820 IU; vitamin D_3, 1,653 IU; vitamin E, 33.1 IU; vitamin K, 4.4 mg; riboflavin, 6.6 mg; niacin, 38.9 mg; pantothenic acid, 22.1 mg; vitamin B_{12}, 0.04 mg; I, 1.1 mg as potassium iodide; Se, 0.30 mg sodium selenite; Zn, 60.6 mg as zinc oxide; Fe, 36.4 mg as ferrous sulfate; Mn, 12.1 mg as manganous oxide; and Cu, 3.6 mg as copper sulfate.

Table 4.3. Effect of increasing standardized ileal digestible (SID) Lys:ME ratio on PRRS viremia and antibody, Exp. 1

Parameter[1]	**SID Lys:ME (g/Mcal)**							***P-value***[2]		
	1.87	2.18	2.49	2.80	3.11	3.41	**SEM**	Diet	dpi	Diet × dpi
PRRSV Ct value[3]										
dpi 7	21.9	21.5	23.5	23.0	21.9	22.0	1.28	0.124	<0.001	0.951
dpi 14	32.8	27.3	30.0	31.4	28.8	32.3				
dpi 21	33.7	32.7	33.7	33.9	32.7	33.3				
dpi 28	37.0	34.2	37.0	36.8	36.6	36.5				
dpi 35	37.0	35.5	35.5	37.0	36.2	37.0				
Genomic PRRSV/mL[4]										
dpi 7	7.33	7.30	6.99	7.01	7.34	7.31	0.71	0.407	<0.001	0.946
dpi 14	3.36	4.92	4.92	4.49	4.60	4.24				
dpi 21	2.40	3.41	3.11	3.76	4.12	3.93				
dpi 28	0.00	2.26	0.00	0.78	0.82	0.88				
dpi 35	0.00	1.15	1.00	0.00	0.96	0.00				
PRRSV S/P ratio[5]										
dpi 7	0.07	0.15	0.04	0.04	0.09	0.04	0.12	0.929	<0.001	0.676
dpi 14	2.22	2.12	2.24	1.90	2.08	2.10				
dpi 21	2.28	2.29	2.30	2.13	2.18	2.24				
dpi 28	2.02	2.24	2.25	2.21	2.13	2.19				
dpi 35	2.19	2.25	2.19	2.13	2.07	2.10				

[1]n=4 pens/diet.
[2]main effect of diet, day post inoculation (dpi) and diet × dpi interaction.
[3]Cycle threshold (Ct), Ct ≥37.0 denotes PRRS negative.
[4]Log_{10} transformation of PRRSV genomic content/mL.
[5]PRRSX3 antibody sample to positive (S/P) ratio, <0.40 denotes PRRS negative.

Table 4.4. Effect of increasing standardized ileal digestible (SID) Lys:ME on growth performance in healthy and PRRSV infected growing pigs, Exp. 1

	SID Lys:ME (g/Mcal)							*P-value*[2]	
Parameter[1]	1.87	2.18	2.49	2.80	3.11	3.41	**SEM**	Linear	Quadratic
Pre-challenge[3]									
Control									
Start BW, kg	36.4	36.4	36.4	36.3	36.4	36.4	0.80	0.962	0.989
ADG, kg	1.02	0.94	0.97	1.01	0.93	1.00	0.03	0.832	0.383
ADFI, kg	1.82	1.79	1.81	1.78	1.75	1.87	0.05	0.893	0.260
G:F	0.558	0.525	0.535	0.570	0.533	0.540	0.013	0.784	0.933
PRRSV									
Start BW, kg	36.8	36.7	37.0	37.0	36.9	37.0	0.76	0.811	0.928
ADG, kg	1.03	0.99	1.05	1.08	1.08	1.01	0.03	0.372	0.148
ADFI, kg	1.88	1.71	1.86	1.88	1.88	1.86	0.04	0.278	0.669
G:F	0.548	0.578	0.565	0.575	0.570	0.548	0.015	0.922	0.110
Challenge[4]									
Control									
Start BW, kg	50.6	49.6	50.0	50.5	49.4	50.4	1.09	0.914	0.675
ADG, kg	1.05	1.11	1.14	1.12	1.13	1.11	0.02	0.069	0.013
ADFI, kg	2.79	2.91	2.85	2.83	2.71	2.87	0.06	0.695	0.891
G:F	0.375	0.383	0.403	0.395	0.418	0.388	0.009	0.039	0.037
PRRSV									
Start BW, kg	52.2	51.6	52.7	53.3	52.9	52.2	0.89	0.563	0.454
ADG, kg	0.70	0.74	0.76	0.86	0.84	0.86	0.04	0.001	0.396
ADFI, kg	2.05	1.99	2.16	2.13	2.12	2.13	0.05	0.068	0.374
G:F	0.343	0.370	0.353	0.408	0.395	0.403	0.014	0.002	0.536
Overall[5]									
Control									
End BW, kg	128.0	130.2	131.5	130.9	130.4	130.4	2.04	0.481	0.336
ADG, kg	1.02	1.06	1.07	1.06	1.07	1.05	0.02	0.387	0.129
ADFI, kg	2.95	3.08	3.00	2.98	2.91	3.04	0.05	0.849	0.955
G:F	0.345	0.343	0.358	0.355	0.368	0.345	0.005	0.128	0.040
PRRSV									
End BW, kg	128.4	129.4	128.5	129.0	128.6	129.7	0.93	0.569	0.788
ADG, kg	0.95	0.97	0.98	0.98	0.99	1.02	0.02	0.011	0.841
ADFI, kg	2.40	2.34	2.46	2.45	2.42	2.47	0.06	0.240	0.964
G:F	0.398	0.415	0.398	0.398	0.410	0.413	0.010	0.500	0.643

[1]n=4 pens/diet.
[2]linear and quadratic orthogonal contrast.
[3]Pre-challenge adaptation period (-14 to 0 days post inoculation (dpi)), all pigs on common diet.
[4]Challenge period (0 to 35 dpi), pigs fed experimental diets.
[5]Overall challenge period (0 dpi to market; control = 76 d, PRRS = 78 ± 2 d).

Table 4.5. Effect of increasing standardized ileal digestible (SID) Lys:ME ratio on carcass characteristics in control and PRRSV infected pigs, Exp. 1

	SID Lys:ME (g/Mcal)							*P-value*[2]	
Parameter[1]	1.87	2.18	2.49	2.80	3.11	3.41	**SEM**	Linear	Quadratic
Control									
Live weight, kg	128.0	130.2	131.5	130.9	130.4	130.4	2.04	0.481	0.336
HCW[3], kg	97.2	100.4	99.9	97.0	98.7	97.9	1.41	0.722	0.405
Dress %	76.1	77.2	76.0	74.1	75.7	75.1	1.28	0.312	0.837
Lean %	53.4	52.0	54.1	54.9	54.2	53.7	0.79	0.205	0.355
Fat thickness, mm	20.76	20.36	20.13	18.38	20.12	20.98	0.58	0.782	0.016
Lean depth, mm	60.19	61.05	62.96	60.31	63.60	63.35	1.24	0.060	0.994
Days to market[4]	76	76	76	76	76	76	-	-	-
PRRSV									
Live weight, kg	128.4	129.3	128.5	129.0	128.6	129.6	0.93	0.581	0.800
HCW[3], kg	97.4	94.7	98.0	96.5	97.7	96.8	1.72	0.755	0.975
Dress %	75.8	73.2	76.2	74.8	75.9	74.7	1.27	0.920	0.918
Lean %	52.5	54.9	54.0	53.7	53.1	53.3	0.96	0.867	0.272
Fat depth, mm	19.71	18.96	20.43	20.57	21.28	21.09	0.78	0.045	0.968
Lean depth, mm	64.70	63.48	63.09	61.91	60.46	60.93	1.72	0.059	0.791
Days to market[4]	80	80	78	78	77	77	0.88	0.004	0.627

[1]n=4 pen/diet.

[2]linear and quadratic orthogonal contrasts.

[3]HCW = hot carcass weight.

[4]All pigs marketed at 76 to 80 d after start of experimental diets and were fed a common control diet.

Table 4.6. PRRS viremia and antibody of control and PRRSV infected pigs, Exp. 2

Parameter[1]	**Control**	**PRRSV**	**SEM**	***P-value***[2]		
				Diet	dpi	Diet × dpi
PRRSV Ct value[3]						
dpi 7	37.0	21.3	1.55	0.115	0.010	<0.001
dpi 14	37.0	28.6				
dpi 21	35.8	34.7				
dpi 28	24.1	36.2				
dpi 49	32.2	37.0				
PRRSV S/P ratio[4]						
dpi 7	0.00	0.87	0.18	<0.001	<0.001	0.005
dpi 14	0.00	1.54				
dpi 21	0.01	1.62				
dpi 28	0.19	1.74				
dpi 49	1.78	1.75				

[1]n=4 pens/diet.
[2]main effect of diet, day post inoculation (dpi) and diet × dpi interaction.
[3]Cycle threshold (Ct), Ct ≥37.0 denotes PRRS negative.
[4]PRRSX3 antibody sample to positive (S/P) ratio, <0.40 denotes PRRS negative.

Table 4.7. Effect of increasing standardized ileal digestible (SID) Lys:ME on growth performance in healthy and PRRSV infected pigs and natural and experimental PRRSV infection, Exp. 2

	SID Lys:ME (g/Mcal)							*P-value*[2]	
Parameter[1]	2.33	2.63	2.94	3.24	3.55	3.85	**SEM**	Linear	Quadratic
Pre-challenge[3]									
Control									
Start BW, kg	23.0	23.1	23.1	22.9	22.8	22.4	0.46	0.299	0.396
ADG, kg	0.48	0.58	0.52	0.46	0.52	0.48	0.04	0.521	0.631
ADFI, kg	1.24	1.36	1.29	1.26	1.33	1.25	0.04	0.886	0.260
G:F	0.388	0.420	0.398	0.363	0.393	0.385	0.026	0.564	0.968
PRRSV									
Start BW, kg	23.3	23.5	23.7	23.9	23.5	22.9	0.57	0.684	0.248
ADG, kg	0.59	0.53	0.58	0.66	0.64	0.62	0.02	0.008	0.808
ADFI, kg	1.43	1.30	1.35	1.41	1.35	1.31	0.04	0.264	0.943
G:F	0.408	0.405	0.435	0.463	0.473	0.473	0.014	<0.001	0.600
Challenge1[4]									
Control									
Start BW, kg	28.2	29.5	28.8	27.9	28.6	27.7	0.71	0.283	0.405
ADG, kg	0.65	0.81	0.87	0.92	0.92	0.92	0.05	<0.001	0.020
ADFI, kg	1.57	1.87	1.76	1.86	1.74	1.72	0.08	0.489	0.039
G:F	0.415	0.433	0.493	0.500	0.533	0.535	0.012	<0.001	0.123
PRRSV									
Start BW, kg	29.7	29.1	30.0	31.1	30.5	29.7	0.73	0.413	0.323
ADG, kg	0.19	0.26	0.43	0.45	0.39	0.49	0.04	<0.001	0.043
ADFI, kg	0.71	0.74	0.90	0.96	0.88	0.98	0.06	0.001	0.262
G:F	0.265	0.345	0.478	0.470	0.443	0.493	0.028	<0.001	0.006
Challenge2[5]									
Natural infection									
ADG, kg	0.58	0.69	0.72	0.82	0.78	0.77	0.03	<0.001	0.003
ADFI, kg	1.49	1.66	1.61	1.79	1.68	1.63	0.06	0.086	0.029
G:F	0.390	0.418	0.443	0.460	0.463	0.473	0.013	<0.001	0.143
Experimental infection									
ADG, kg	0.55	0.58	0.72	0.78	0.73	0.75	0.03	<0.001	0.007
ADFI, kg	1.23	1.23	1.46	1.54	1.41	1.50	0.05	<0.001	0.037
G:F	0.445	0.468	0.495	0.503	0.515	0.500	0.018	0.011	0.147
Overall[6]									
Control									
End BW, kg	116.2	130.2	123.4	130.7	131.5	125.2	2.49	0.016	0.006
ADG, kg	0.88	1.01	0.95	1.03	1.03	0.98	0.02	0.002	0.003
ADFI, kg	1.66	1.85	1.78	1.97	1.85	1.80	0.07	0.12	0.032
G:F	0.530	0.548	0.535	0.520	0.560	0.543	0.016	0.529	0.812
PRRSV									
End BW, kg	121.4	120.9	129.3	129.9	129.7	132.5	2.62	0.002	0.473
ADG, kg	0.90	0.90	0.97	0.97	0.97	1.01	0.02	0.001	0.712
ADFI, kg	1.44	1.41	1.66	1.72	1.61	1.69	0.05	<0.001	0.048
G:F	0.625	0.638	0.585	0.563	0.610	0.598	0.016	0.091	0.081

[1]n=4 pens/diet.
[2]Linear and quadratic orthogonal contrast.
[3]Pre-challenge adaptation period (-14 to 0 dpi), all pigs on common diet.
[4]Challenge period 1 (0 to 21 dpi), pigs fed experimental diets.
[5]Challenge period 2 (0 to 49 dpi), Control barn naturally infected with PRRSV after 21 dpi, pigs fed experimental diets.
[6]Overall challenge period (0 dpi to market); control pigs naturally infected after 21 dpi, PRRSV pigs experimentally infected at 0 dpi.

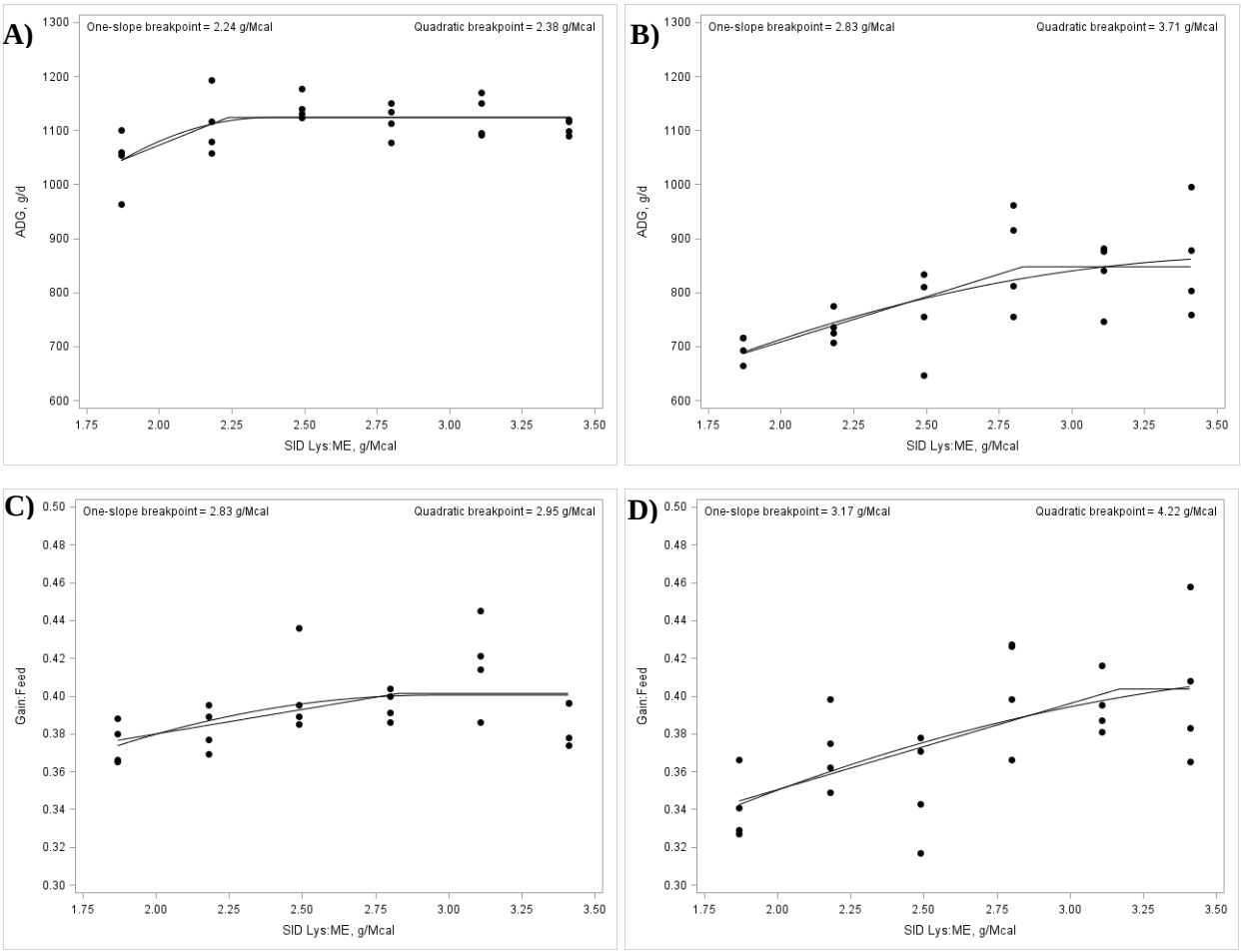

Figure 4.1. Data points represent treatments means from 4 pens per experimental diet per health status. One-slope straight broken-line and quadratic broken-lines were fitted for maximum ADG (A-B) and G:F (C-D) expressed as a function of standardized ileal digestible (SID) Lys:ME (g SID Lys per Mcal ME) over a 35 d growth period in control (A,C) and PRRSV (B,D) infected pigs, respectively. **A**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 2.24 g/Mcal (Y plateau = 1123.6 ADG; slope below requirement = −216.1; r^2 = 0.38). The quadratic broken-line model resulted in a SID Lys:ME requirement of 2.38 g/Mcal (Y = 1123.6 − 302.5(2.38 − g SID Lys/Mcal)^2; r^2 = 0.38). **B**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 2.83 g/Mcal (Y plateau = 847.4 ADG; slope below requirement = −167.2; r^2 = 0.47). The quadratic broken-line model yielded a SID Lys:ME requirement of 4.71 g/Mcal (Y = ; r^2 = 0.45); however, this predicted requirement is outside the range of the diets tested. **C**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 2.83 g/Mcal (Y plateau = 0.401 G:F; slope below requirement = -0.026; r^2 = 0.23). The quadratic broken-line model yielded a SID Lys:ME requirement of 2.95 g/Mcal (Y = ; r^2 = 0.25). **D**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 3.17 g/Mcal (Y plateau = 0.404 G:F; slope below requirement = −0.046; r^2 = 0.41). The quadratic broken-line model projected a SID Lys:ME requirement of 4.22 g/Mcal (Y = 4.22 – 0.013(4.22 − g SID Lys/Mcal)^2; r^2 = 0.41); however, the predicted requirement is outside the range of the experimental diets.

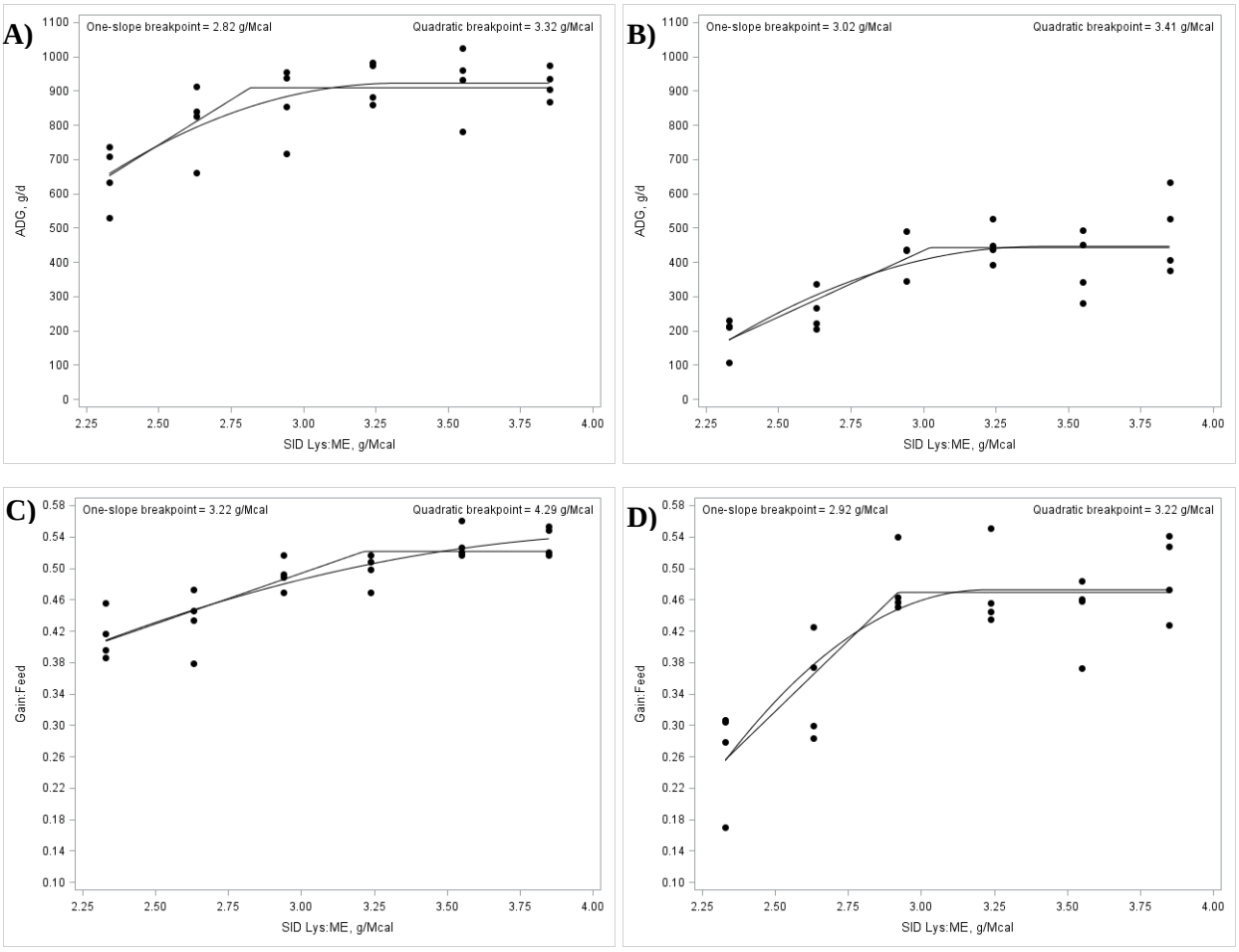

Figure 4.2. Data points represent treatment means from 4 pens per experimental diet per health status. One-slope straight broken-line and quadratic broken-lines were fitted for maximum ADG (A-B) and G:F (C-D) expressed as a function of standardized ileal digestible (SID) Lys:ME (g SID Lys per Mcal ME) over a 21 d growth period in control (A,C) and PRRSV (B,D) infected pigs, respectively. **A**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 2.82 g/Mcal (Y plateau = 908.8 ADG; slope below requirement = −526.7; r^2 = 0.59). The quadratic broken-line model resulted in a SID Lys:ME requirement of 3.32 g/Mcal (Y = 921.9 − 267.2(3.32 − g SID Lys/Mcal)^2; r^2 = 0.61). **B**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 3.02 g/Mcal (Y plateau = 442.2 ADG; slope below requirement = −387.3; r^2 = 0.65). The quadratic broken-line model yielded a SID Lys:ME requirement of 3.41 g/Mcal (Y = 445.4 − 235.8(3.41 − g SID Lys/Mcal)^2; r^2 = 0.63). **C**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 3.22 g/Mcal (Y plateau = 0.521 G:F; slope below requirement = −0.129; r^2 = 0.74). The quadratic broken-line model yielded a SID Lys:ME requirement of 4.29 g/Mcal (Y = 0.544 – 0.036(4.29 – g SID Lys/Mcal)^2; r^2 = 0.78); however, this predicted value is outside the range of the experimental diets tested. **D**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 2.92 g/Mcal (Y plateau = 0.469 G:F; slope below requirement = −0.361; r^2 = 0.72). The quadratic broken-line model projected a SID Lys:ME requirement of 3.22 g/Mcal (Y = 0.472 – 0.272(3.22 − g SID Lys/Mcal)^2; r^2 = 0.69).

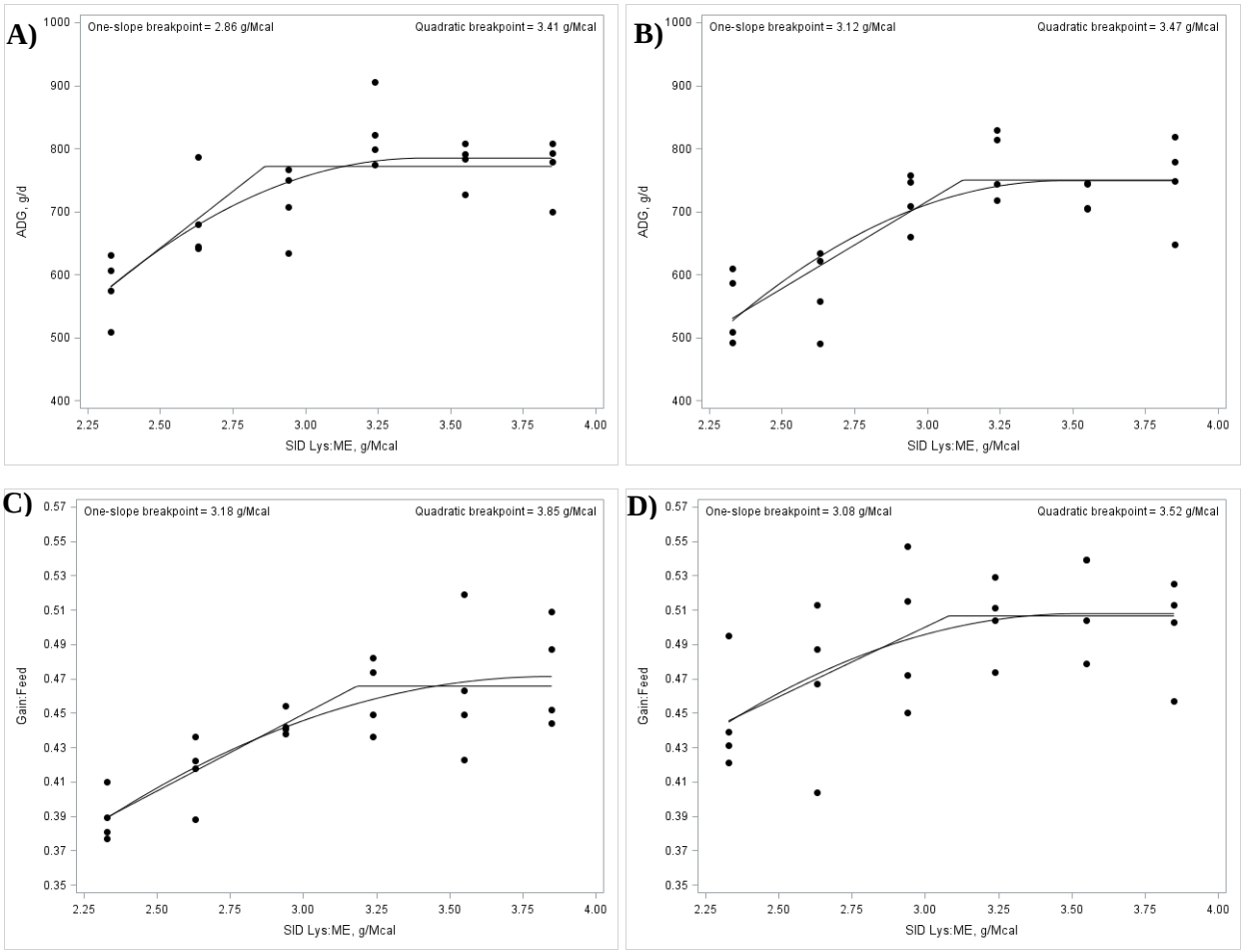

Figure 4.3. Data points represent treatments means from 4 pens per experimental diet per health status. One-slope straight broken-line and quadratic broken-lines were fitted for maximum ADG (**A-B**) and G:F (**C-D**) expressed as a function of standardized ileal digestible (SID) Lys:ME (g SID Lys per Mcal ME) over a 49 d growth period in pigs that experienced a natural (A,C) or experimental (B,D) PRRSV infection, respectively. **A**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 2.86 g/Mcal (Y plateau = 771.3 ADG; slope below requirement = −360.0; r^2 = 0.62). The quadratic broken-line model resulted in a SID Lys:ME requirement of 3.41 g/Mcal (Y = 784.4 – 176.4(3.41 − g SID Lys/Mcal)^2; r^2 = 0.67). **B**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 3.12 g/Mcal (Y plateau = 749.6 ADG; slope below requirement = −276.8; r^2 = 0.71). The quadratic broken-line model yielded a SID Lys:ME requirement of 3.47 g/Mcal (Y = 748.6 – 171.3(3.47 – g SID Lys/Mcal)^2; r^2 = 0.67); however, this predicted requirement is outside the range of the diets tested. **C**) The one-slope straight broken-line model yielded a SID Lys:ME requirement of 3.18 g/Mcal (Y plateau = 0.466 G:F; slope below requirement = -0.089; r^2 = 0.64). The quadratic broken-line model yielded a SID Lys:ME requirement of 3.85 g/Mcal (Y = 0.471 – 0.036(3.85 – g SID Lys/Mcal)^2; r^2 = 0.65). **D**) The one-slope straight broken-line model resulted in a SID Lys:ME requirement of 3.08 g/Mcal (Y plateau = 0.506 G:F; slope below requirement = −0.081; r^2 = 0.36). The quadratic broken-line model projected a SID Lys:ME requirement of 3.52 g/Mcal (Y = 0.508 – 0.044(3.52 − g SID Lys/Mcal)^2; r^2 = 0.36).

CHAPTER 5. GENERAL DISCUSSION

Almost inevitably, pigs will face an immunological challenge in their life, whether from vaccinations or live pathogen exposure. From a pathogen standpoint, one of the most prevalent disease agents in the U.S. swine herd is porcine reproductive and respiratory syndrome virus (**PRRSV**). It has been reported that more than 45% of U.S. nursery sites and 50% of U.S. finisher sites have had a PRRSV incidence (USDA, 2015), and 70% of the US swine herd have tested positive for PRRS antibody (NAHMS, 2009). Similarly, *Brachyspira hyodysenteriae* (**Bhyo**) affects pigs worldwide, with a prevalence of up to 75% in some worldwide herds (Suh and Song, 2005; Alvarez-Ordonez et al., 2013; Dors et al., 2015), and is a reemerging pathogen in U.S. swine herds (Burrough, 2017). Even though PRRSV and Bhyo are highly prevalent, infected pigs are more often than not fed similarly to healthy pigs based on NRC (2012) requirement guidelines. This requirement assumption may be false and based on current knowledge of immune system requirements; it has been hypothesized that nutrient and amino acid (**AA**) requirements may be different to support an activated immune response (Reeds et al., 1994; Reeds and Jahoor, 2001). Further, reduced tissue accretion rates and performance as a result of pathogen challenges (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017) suggest an alteration in nutrient utilization and resource allocation (Rakhshandeh et al., 2010; Rauw, 2012). This metabolic shift and nutrient reallocation is critical for efficient and effective immune response and resolution through the support of increased proliferation of immune cells and proteins; however, if overzealous and prolonged, it can impede skeletal muscle growth (Williams et al., 1997a, b, c; Schweer et al., 2017). Further, alterations in endogenous secretions that include digestive enzymes, mucins, sloughed cells, peptides, and free AA and can be altered

by the physiological state of pigs (Adeola et al., 2016); therefore, changes in basal endogenous AA losses (**BEL**) can reflect changes in AA requirements and may be impacted by different disease states in growing pigs.

Reeds and Jahoor (2001) observed that the general cytokine response and peak nitrogen (**N**) loss during different infections or trauma (i.e., surgery, injury, viral infection, or bacterial infection) is uniform, and the eventual deleterious effects of these different insults are primarily a reflection of the duration of the response rather than its initial metabolic magnitude. Although the general metabolic response to pathogens may be similar across diseases, the impact on feed intake and nutrient absorption may differ between site specific infection (i.e., enteric vs. respiratory). However, data is lacking in verifying these difference or similarities as they relate to digestive function. In particular, to what degree BEL are altered, and when applied to apparent ileal digestibility (**AID**) values, how this would alter the calculated standardized ileal digestibility (**SID**) of AA. Having SID AA values are more accurate than AID or total AA values and allows for more accurate diet formulation of AA during health challenges. Therefore, the overall objective of this dissertation was to determine how two different pathogens impact BEL and AA digestibility values and to determine if altered dietary AA formulations could improve pig performance during a disease challenge. To address this overarching objective, three research experiments (Chapters 2, 3, and 4) were conducted.

The objective of the first research chapter (Chapter 2), was to examine the interaction of dietary soybean meal (**SBM**) and PRRSV on ileal and total tract digestibility of AA and to determine the extent to which PRRSV may alter BEL of AA. This was achieved using T-cannulated grower pigs in which AID, BEL, and SID coefficients were determined at 7-8 days post-inoculation (**dpi**) (close to peak viremia) and again at 18-19 dpi (approximately the time of

seroconversion). Increasing SBM during a health challenge in growing pigs has been reported to improve aspects of growth and viral clearance (Boyd et al., 2010; Rochell et al., 2015); however, the mechanism is unclear. Therefore, we hypothesized that a potential benefit of increased SBM during a PRRSV challenge is due to increased SID of AA. Previous work suggested that PRRSV did not alter AID of AA (Schweer et al., 2016); however, BEL have not been determined and SID calculated for pigs during a PRRSV challenge. Data presented herein (Chapter 2) compared diets with increasing SBM from 10% to 29% (i.e., meeting all essential AA needs from SBM) and this expectedly increased crude protein from 13% to 18.5%. To keep dietary AA profiles similar, crystalline AA were increased in the low SBM diet to normalize Lys (1.10 and 1.12%, respectively) and to maintain the ratio of Lys to Thr, Trp, Met, and Val. In a 2 × 2 + 2 factorial, complete randomized design, 48 kg BW pigs were fitted with a T-cannula and inoculated with live PRRSV or a sham inoculum for a 19 d experimental period. Further, a cohort of pigs were fed a nitrogen-free diet (**NFD**) diet to determine BEL. Two collection periods were undertaken to capture digestibility differences during peak viremia and seroconversion phases of the disease. Interestingly, there was a tendency for a SBM by PRRSV interaction for AID of Arg and SID of Pro only at dpi 7-8. Arginine AID was not different during PRRSV challenge in pigs fed high SBM, while Arg AID increased with PRRSV infection in low SBM fed pigs. Proline SID was decreased due to PRRSV infection similarly in both high and low SBM diets, and increased in low SBM, regardless of challenge. No other interactions for AID or ATTD values were detected in the study, suggesting increased N or AA digestibility may not be the mechanism for SBM to be beneficial during PRRSV challenge.

Although PRRSV is a significant swine pathogen, there is limited data available on how it impacts nutrient, AA, and energy digestibility. At 21 dpi in nursery pigs, AID of AA were not

impacted by PRRSV (Schweer et al., 2016). In Chapter 2, except for a tendency to reduce Thr AID at 7-8 dpi, PRRSV did not alter AA AID at either collection period. At 7-8 dpi, PRRSV challenge reduced AID of DM and GE, but N was not different compared to the non-challenged control pigs. The SID values were calculated by adjusting AID values for BEL, which were determined using a NFD. To the author's knowledge this is one of a limited number of studies that have attempted to determine BEL values in pigs using a live pathogen, and the first to our knowledge to have used a respiratory/systemic pathogen like PRRSV. During enteric infections, it is anticipated that ileal BEL will increase; however, during a respiratory challenge, it was hypothesized that ileal BEL would not change. At 7-8 dpi, BEL of Arg, Ala, and Pro were reduced, and ileal N tended to be reduced by PRRSV infection, while total tract N was increased by PRRSV infection. At 18-19 dpi, there was only a tendency for BEL of Thr to be increased by PRRSV and no other differences were noted. When SID were calculated, PRRSV reduced Arg, Gly, and Pro SID and tended to reduce Ala and Ser SID at 7-8 dpi; however, at 18-19 dpi, only Pro SID was reduced.

Next, we set out to examine how an enteric specific pathogen insult may alter AA digestibility and BEL in growing pigs. A small body of research suggests that pathogenic bacterial enteric agents such as *Salmonella* Typhimurium and enterotoxigenic *Escherichia coli* can reduce AID of N and AA (Heo et al., 2010; Lee, 2012). Similarly, Lee (2012) reported increased BEL, and therefore, reduced SID of AA in nursery and growing pigs due to *Salmonella* Typhimurium challenge. Both these Gram-negative bacterial pathogens impact the small intestine; however, alterations in digestibility during a strict colitis challenge are not known. Therefore, a second experiment (Chapter 3) was performed to determine the impact of Bhyo on AID, BEL, and SID values. Although Bhyo, the causative agent of swine dysentery and colitis, is

a pathogen that impacts pigs worldwide, little is known about how it impacts metabolism. Again, T-cannulated grower pigs (63 kg BW) were challenged with or without Bhyo and were fed either a complete diet or NFD. Expectedly, Bhyo reduced ATTD of DM, N, and GE. Interestingly, AID of Gly was increased by Bhyo and no other nutrient, AA, or energy AID differences were reported. It was anticipated that BEL of mucin-related AA (i.e., Thr, Ser, and Cys) would be increased in the small intestine. These AA make up approximately 50% of mucins and Bhyo can cause mucin hypersecretion in the colon (Wilberts et al., 2014; Quintana-Hayashi et al., 2015). Similar to PRRSV in Chapter 2, BEL of Pro was reduced by Bhyo. There was also a tendency for BEL of Arg, Trp, and Gly to be reduced by Bhyo. Although these ileal BEL results were unexpected based on data from *S.* Typhimurium challenge (Lee, 2012), it is reasonable when considering that Bhyo strictly infects the large intestine and small intestine function remains unaffected (Argenzio et al., 1980). After adjusting AID with BEL, SID of N, Arg, Lys, Ala, Gly, Pro, and Ser were reduced due to the 13 dpi Bhyo challenge and the SID of Thr tended to be reduced.

After investigating how respiratory/systemic (PRRSV) and colitis (Bhyo) models may modulate small intestinal nutrient disappearance, we also assessed if these two pathogenic agents modulated hindgut function by quantifying differences in hindgut disappearances of nutrients and energy. In Chapter 2, around peak viremia (dpi 5-8), hindgut disappearance of DM and GE increased by 21% and 23%, respectively, in PRRSV challenged pigs; however, no differences were noticed at seroconversion (dpi 16-19). This may be reflective of an increase in microbial density of the hindgut which can improve growth performance and reduce lung lesions during a PRRSV challenge (Niederwerder et al., 2016). When hindgut disappearance was calculated from 9 to 13 dpi in the Bhyo challenge (Chapter 3), there was an increase in the net appearance of

DM, N, and GE by two-fold, 58-fold, and nine-fold, respectively. Increased appearance of nutrients and energy in the hindgut of Bhyo pigs was somewhat expected due to hemorrhagic diarrhea, increased mucin secretion, and cell sloughing associated with the swine dysentery disease (Albassam et al., 1985; Quintana-Hayashi et al., 2015). Taken together, although hindgut N disappearance does not contribute to overall N balance (Just et al., 1981), hindgut energy disappearance can contribute to overall energy balance and systemic immune function (Rérat, 1978; Spiljar et al., 2017).

In Chapter 4, two experiments were conducted to test whether increasing dietary Lys relative to metabolizable energy (**ME**) would improve growth and/or feed efficiency in PRRSV infected pigs. When feed intake can be altered due to dietary energy density or the pig's physiological state (i.e., disease), it may be beneficial to express AA in relation to dietary energy (Lewis, 2002). Although Lys efficiency does not appear to be impacted by a sanitary challenge in young and growing pigs (Williams et al., 1997a, b), growth performance and feed efficiency are improved when dietary Lys is increased (Williams et al., 1997b, c; Kahindi et al., 2013). Lysine is the first limiting AA for growth in healthy pigs and in poultry, and Lys use by the immune system increases during lipopolysaccharide (**LPS**) challenge in order to support immune protein synthesis (Klasing and Calvert, 1999). In Chapter 2 we showed that PRRSV did not alter SID of Lys. Therefore, increasing dietary Lys relative to ME intake would result in an increase in body Lys pools providing more Lys for protein synthesis and increased growth. Thus, the objective of Chapter 4 was to determine the optimal SID Lys to ME ratio (**Lys:ME**) of diets in PRRSV challenged pigs at two ages using breakpoint analysis. We hypothesized that growth and feed efficiency would increase linearly with increased dietary Lys:ME.

In the first experiment, 384 pigs (50 kg BW) were group penned (8 pigs/pen) in two separate barns; one barn was challenged with PRRSV while the control barn received a sham inoculation. Pigs were fed one of six diets containing 1.87, 2.18, 2.49, 2.80, 3.11, or 3.41 Lys:ME ratio, representing 80, 90, 100, 110, 120, and 130% of NRC (2012) requirement, respectively (n = 4 pens/trt), during a 35 d growth period. The PRRSV challenged pigs had confirmed viremia and the control pigs remained negative for PRRSV over the challenge period. Growth and feed efficiency increased quadratically in control pigs and linearly in PRRSV infected pigs with increasing Lys:ME during the 35 d challenge period. Feed intake was not affected by diet in control pigs; however, it tended to increase in PRRSV pigs as Lys:ME increased. Using breakpoint analysis, it was determined that PRRSV increased Lys:ME for optimal ADG by 21% to 36% and G:F by 11% to 30%; however, using a quadratic model, a plateau was not achieved suggesting the requirement could be higher than the diets tested.

Using a similar design, a second experiment was conducted to determine the impact PRRSV has on Lys;ME requirement for growth and feed efficiency in 25 kg BW pigs. Pigs were again split across two barns, one infected with PRRSV and the other sham inoculated. This time, pigs were fed one of six diets containing 2.33, 2.63, 2.94, 3.24, 3.55, and 3.85 Lys:ME, representing 80, 90, 100, 110, 120, and 130% of NRC (2012) requirement, respectively (n = 4 pens/trt), for a 49 d growth study. Between 21 and 28 dpi, control pigs became naturally infected with PRRSV as determined by viremia and serology. Therefore, ideal Lys:ME for growth and feed efficiency was determined for the PRRSV and control pigs from 0 to 21 dpi, and the ideal Lys:ME was also determined for natural and experimental PRRSV infection over the 49 d growth period. Growth and feed efficiency increased linearly with increasing Lys:ME in PRRSV infected pigs and control pigs. Feed intake increased quadratically in control pigs and linearly in

PRRSV infected pigs. Similar to the first experiment, PRRSV increased Lys:ME for optimal ADG by 3% to 7% and G:F by 9% to 25%. Again, a plateau was not achieved in the quadratic model suggesting the requirement could be higher than the experimental diets tested. When comparing natural and experimental infection, growth and feed efficiency increased linearly with increasing Lys:ME in both groups. Interestingly, the optimal Lys:ME for growth and feed efficiency was similar (~110% of NRC (2012) requirement) between natural and experimental infection.

The data reported in this dissertation have provided a better understanding of nutrient requirements, specifically AA requirements, during PRRSV and Bhyo infection, and how to better formulate diets during health challenges. Both PRRSV and Bhyo had minimal impact on AID of nutrients and AA and BEL of AA. As PRRSV is not an enteric infection and Bhyo does not impact the small intestine, these data are not unexpected. Similarly, SID of AA were not greatly impacted by PRRSV, and while Bhyo reduced the SID of some AA, all SID values were above 90%. Total tract N loss was increased by PRRSV which is likely related to the hindgut microbiota. The microbial density and diversity in the hindgut may significantly impact the outcome of different respiratory pathogens (Schachtschneider et al., 2013; Niederwerder et al., 2016). Also, from this dissertation, increasing dietary Lys:ME to 110% to 120% of NRC (2012) improves growth and feed efficiency in PRRSV infected pigs, and natural and experimental PRRSV infection have similar Lys:ME requirements.

In PRRSV pigs there was an increase in total tract N loss which is likely related to the hindgut microbiota. In previous studies, pigs with increased microbial density and diversity in the hindgut had improved performance during PRRSV infection (Niederwerder et al., 2016), and reduced coughing and lung lesions in *Mycoplasma hyopneumoniae* infected pigs

(Schachtschneider et al., 2013). These data suggest that increased microbial density in the hindgut can improve systemic immunity and benefit the host. A potential mechanism of how the hindgut microbiota improves systemic immunity is by contributing to overall host energy balance (Spiljar et al., 2017). Although N and AA absorption in the hindgut is negligible, and hindgut N disappearance does not contribute to overall N balance (Sauer et al., 1979; Just et al., 1981), hindgut fermentation and volatile fatty acid production can contribute to maintenance energy, improving energy balance (Rérat, 1978; Dierick et al., 1989). It is reasonable to consider then that, although hindgut energy utilization can contribute to overall energy balance, a reduced energy and N disappearance, or net appearance as is seen in Bhyo challenge, can negatively contribute to overall energy and N balance and should be explored further.

Minimal differences in SID of AA were noted due to PRRSV, and although the SID of some AA were reduced due to Bhyo, SID values were above 90%. This suggests that reduced performance is not likely due to reduced SID of AA, but more likely attributed to reduced feed intake. We have reported with PRRSV (Schweer et al., 2016; Schweer et al., 2017), porcine epidemic diarrhea virus (**PEDV**) (Schweer et al., 2016; Curry et al., 2017), PRRSV and PEDV co-challenge (Schweer et al., 2016) and *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* co-challenge (Helm et al., 2018), that feed intake is reduced 6-30% in growing pigs. Physiologically, this could be a result of increased pro-inflammatory cytokines during a health challenge (Johnson, 1997), signaling the hypothalamus and leading to a decrease in voluntary feed intake (Plata-Salaman and Borkoski, 1993; Plata-Salamán, 1998). This is thought to be a coping mechanism during infection to limit nutrient availability to the pathogen (Plata-Salamán, 1998; Johnson, 2002). It has been determined that reduced feed intake is necessary during disease. In mice challenged with *Listeria monocytogenes* and force-fed to the same feed

intake as healthy, non-infected mice, mortality was 93% (Murray and Murray, 1979). Similarly, when pigeons were challenged with *Candida albicans* and force-fed to the same intake as healthy controls, mortality and morbidity were 50% and 100%, respectively (Tsat et al., 1994). Force-feeding to ad libitum levels appears unwarranted; however, there may be ways to compensate for decreased feed intake and to stimulate appetite that can be explored. We know that pigs eat to meet their energy demands (Nyachoti et al., 2004). Therefore, it would seem plausible to increase dietary nutrient as a ratio to energy, either by increasing dietary nutrients while keeping energy constant or by decreasing dietary energy. This would provide more nutrients, specifically essential AA and conditionally essential AA, to the pig and compensate for decreased feed intake. Similarly, AA requirement studies in health challenged pigs generally focus on only a specific AA. In rats with dextran sodium sulfate induced colitis, increasing dietary Thr, Cys, Pro, and Ser restored mucin AA composition to that of controls, and promoted growth of commensal gut microbiota, but did not improve weight gain (Faure et al., 2006). Therefore, increasing multiple, or all, dietary AA instead of a single AA can prove beneficial during health challenges and should be a primary area of exploration.

Disease stress is metabolically different from starvation stress. During starvation, lipid stores are mobilized to generate energy to conserve body protein and AA. In contrast, during disease stress, body proteins are catabolized at an increased rate and body fat stores are not readily mobilized (Beisel and Wannemacher, 1980). In rodents and poultry, reduced feed intake accounts for 40% of total N loss during a health challenge (Reeds and Jahoor, 2001) and 40-70% of BW losses (Klasing, 1988; Klasing and Calvert, 1999). The remaining reductions are thought to be from increased lean tissue turnover as a result of higher rates of proteolysis verses protein synthesis (Bruins et al., 2000; Bruins et al., 2003; Orellana et al., 2004). This is thought to

provide adequate AA to meet increased protein and energy demands of the activated immune system (Bruins et al., 2003; Melchior et al., 2004; Capozzalo et al., 2017).

Another important aspect of resource reallocation is the metabolic flexibility of cells and tissue that allows for the immunometabolic governance of the immune response. The immune system relies heavily on glucose to generate energy, and when the immune system becomes activated, several immune cell types rely on a metabolic switch favoring glycolysis rather than oxidative phosphorylation for ATP (Pearce and Pearce, 2013; O'Neill, 2014). This is evidenced by substantial increases in circulating lactate and glucose utilization in LPS challenged growing pigs (Kvidera et al., 2017). Further, Gln is essential for immune cell activation, can serve as a fuel substrate for immune cells, and is necessary for lymphocyte protein synthesis (Waithe et al., 1975; Calder, 1995; Maciolek et al., 2014). As whole body protein accretion is reduced in health challenged pigs (Williams et al., 1997a, b, c; Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017), it is assumed that skeletal muscle provides AA for glycolytic fuel and proteins for the immune response.

Lipid deposition is also decreased during a health challenge (Williams et al., 1997c; Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017), which coupled with decreased skeletal muscle, reduces energy stores in the body leading to reduced energy available for the immune system. This increases the importance of providing energy sources (glucogenic and ketogenic AA, glucose, lipids) in the diet. A pair-fed model can be utilized to better quantify the metabolic cost of the immune response. With this model, healthy pigs are fed to the same level of infected pigs, negating the impact of feed intake. In a LPS model, feed efficiency and N retention were reduced and protein degradation rate was increased 14%, 14%, and 13%, respectively, from pair-fed controls (Daiwen et al., 2008). Using the same LPS pair-fed model,

Dritz et al. (1996) and Hevener et al. (1999) reported no difference in feed efficiency between LPS and pair-fed pigs. Interestingly, to our knowledge, a pair-fed model has not been used for swine industry applicable live pathogens to study changes in tissue accretion and AA partitioning and warrants future investigation.

As noted in Chapter 1, attention has been given to sulfur-containing AA (**SAA**), Trp, Thr, and Lys when determining AA requirements in health challenged pigs because of their importance to the immune response or growth. Sulfur-containing AA are abundant in inflammatory proteins and peptides and a critical for maintaining oxidative status (Grimble, 1994; Grimble, 2006). Tryptophan serves as a precursor for serotonin that mediates the stress response and feed intake (Le Floc'h and Seve, 2007) and can be metabolized by immune cells (Maciolek et al., 2014). Threonine and Lys are two of the most limiting AA in commercial swine diets and are both needed for protein synthesis. Data provided in this dissertation would suggest that attention should also be given to Gly and Pro. Glycine and Pro are both essential for piglets and can become essential in adults when facing stressful conditions (Wu, 2013). Both are abundant in sow colostrum and milk (Wu and Knabe, 1994), and are essential in young pigs to maximize protein synthesis and N retention (Ball et al., 1986; Wang et al., 2014). Similarly, Gly is conditionally essential as a N donor in young pigs (Goodband et al., 2014) and assumedly in stressed (disease) states as well. In young pigs, Pro can also be interconverted to Arg or ornithine in the small intestine and is a precursor for Glu synthesis, potentially increasing the requirement for Pro (Bertolo et al., 2003). Apart from acting as a precursor for other AA, Pro and Gly are abundant in collagen (Eyre and Muir, 1975), which acts as a structural scaffold in tissues and is a primary component of wound healing (Brett, 2008). During PRRSV, collagenase activity is increased in the lung (Girard et al., 2001). In Bhyo infection, colonocytes can detach and slough

away, increasing the need for collagen for colonic wall reassembly (Hesp et al., 1984; Albassam et al., 1985). Together, these data suggest an increased need for Pro and Gly during different types of infection.

Surprisingly, the addition of supplemental branched-chain AA (**BCAA**) Leu, Ile, and Val or their metabolites to health-challenged pig diets has been relatively unexplored. Branched-chain AA, specifically Leu, can stimulate protein synthesis through signaling of the mammalian target of rapamycin (**mTOR**) pathway (Columbus et al., 2015). In addition, all three BCAA are required for immune cell growth and proliferation (Glassy and Furlong, 1981; Calder, 2006). Based on the physiological significance of BCAA two experiments were conducted to determine if increasing dietary Leu would improve the outcome of LPS stimulated pigs. These two separate studies demonstrated that increasing dietary Leu 150% or 200% above NRC (2012) did not improve N retention or protein deposition in 10 kg or 15 kg BW pigs (Rudar et al., 2016; Rudar et al., 2017). Although the BCAA are important for protein synthesis and immune function, excess Leu can antagonize both Val and Ile by increasing their oxidation and thus impairing growth (Oestemer and Hanson, 1973; Gatnau et al., 1995), providing a potential reason why increasing Leu did not prove beneficial during LPS challenge. Interestingly, some BCAA metabolites, like β-hydroxy-β-methylbutyrate (**HMB**), can improve weaning weight in suckling pigs (Nissen et al., 1994) and reduce skeletal muscle proteolysis in rats (Holecek et al., 2009). Additionally, increasing HMB in cultured sheep and chicken immune cells increased immune cell proliferation (Nissen and Abumrad, 1997), and can improve pulmonary function and reduce inflammation in chronic obstructive pulmonary disease patients (Hsieh et al., 2006). Interestingly, HMB has not been supplemented to health challenged pigs and could be an opportunity for further research.

In addition to increased collagenase activity in the lung of PRRSV infected pigs, Girard et al. (2001) also reported an increase in lung matrix metalloproteinases activity. In experimentally induced colitis models in mice, these metalloproteinases are upregulated as well (Garg et al., 2009), suggesting they are likely increased in Bhyo challenged pigs. Matrix metalloproteinases are zinc-dependent enzymes secreted by resident and inflammatory cells that degrade extracellular matrix which provides physical scaffolding for cellular constituents and initiates important biochemical signals to maintain tissue homeostasis structural support (Van Wart and Birkedal-Hansen, 1990; Frantz et al., 2010). Increased dietary zinc can maintain collagen levels and protect against lipid peroxidation in the liver of ethanol-fed rats (Cabré et al., 1995). Increasing dietary zinc may also be beneficial in reducing collagen degradation in pigs. Thus, in addition to AA, dietary micronutrient requirement changes during disease and inflammation challenges in pigs warrants further investigation.

In summary, the body of work presented in this dissertation has shown that ileal digestibility of nutrients, energy, and AA and ileal endogenous AA losses are not greatly impacted by respiratory disease or colitis in growing pigs. Additionally, during a PRRSV challenge, ideal growth and feed efficiency occur when dietary Lys:ME is 110% or 120% of NRC (2012) requirement. These findings are significant, as they can be adopted by the swine industry to aid in the better formulation of diets for health compromised pigs. However, we have also debunked a few assumptions in that increasing dietary SBM during a PRRSV challenge does not improve the digestibility of AA, suggesting an alternative method by which SBM can improve the outcome of PRRSV infected pigs. More research needs to be done with regard to dietary nutrient and AA density and if increasing one or multiple dietary AA can be beneficial during disease to mitigate production losses associated with disease. Moreover, increasing other

dietary components, like micronutrients, could be beneficial during disease to improve resolution and recovery from disease.

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APPENDIX. OTHER PUBLICATIONS AND ABSTRACTS

Peer Reviewed Publications

Pearce, S.C., **W. Schweer**, K. Schwartz, K.J. Yoon, S. Lonergan, and N. Gabler. 2016. Pig jejunum protein profile changes in response to a porcine epidemic diarrhea virus challenge. J. Anim. Sci. 94 Supplement 3: 412-415 doi:10.2527/jas.2015-9815

Invited Presentations

The XVIII AMENA Congress *"A literature review and evaluation of sub-therapeutic growth promoting antibiotic alternatives"* Oct. 18, 2017 Puerto Vallarta, Jalisco, Mexico

Allen D. Leman Conference *"A literature review and evaluation of sub-therapeutic growth promoting antibiotic alternatives",* Sept. 18, 2017 St. Paul, MN

World Pork Expo *"Sub-therapeutic growth promoting antibiotic alternatives*" June 7, 2017, Des Moines, IA.

Proceedings and Conference Papers

W.P. Schweer, O.F. Mendoza, C.M. Shull, J. Lehman, A.M. Gaines, K.J. Schwartz, and N.K. Gabler (2018) Increased Lysine:Metabolizable energy ratio improves grower pig performance during a porcine reproductive and respiratory syndrome virus challenge. ASAS-ADSA Midwest Sectional Meeting, Omaha, NE. March 12-14, Abstract #45

E.T. Helm, **W.P. Schweer**, C.M. De Mille, S.M. Curry, and N.K. Gabler (2018) Understanding the Performance-enhancing mode of action of in-feed subtherapeutic antibiotics in nursery pigs. ASAS-ADSA Midwest Sectional Meeting, Omaha, NE. March 12-14, Abstract #135

K.M. Olsen, N.K. Gabler, C.J. Rademacher, K.J. Schwartz, **W.P. Schweer**, and J.F. Patience (2018) The effects of group size and subtherapeutic antibiotic alternatives on the performance of nursery piglets: a model for feed additive evaluation. ASAS-ADSA Midwest Sectional Meeting, Omaha, NE. March 12-14, Abstract #284

N.K. Gabler, A. Ramirez, **W.P. Schweer** (2017) Efficacy of sub-therapeutic antibiotic alternatives. James D. McKean Swine Disease Conference, Ames, IA. Nov. 2-3

W. Schweer, A. Ramirez, N. Gabler (2017) Alternatives to in-feed antibiotics for nursery pigs. The XVIII AMENA Congress, Puerto Vallarta, Jalisco, Mexico Oct. 17-21

N.K. Gabler and **W.P. Schweer** (2017) Alternatives to in-feed antibiotics for nursery pigs. Minnesota Nutrition Conference, Mankato, MN. Sept. 20-21

C.L. Loving, Z.F. Olson, **W.P. Schweer**, and N.K. Gabler (2017) Infection with Porcine Reproductive and Respiratory Syndrome virus (PRRSv) impacts immune cell metabolism without evidence of cell-mediated immune response. Journal of Immunology 198 Supplement 1:226.7

W.P. Schweer, J.F. Patience, E.R. Burrough, N. Gabler (2017) Impact of PRRSV on digestibility and endogenous losses in pigs fed high or low soybean meal diets. ASAS-ADSA Midwest Sectional Meeting, Omaha, NE. March 13-15, Abstract #193

W.P. Schweer, J.F. Patience, K. Schwartz, D. Linhares, C. Rademacher, H. Allen, C. Loving, A. Ramirez, N.K. Gabler (2017) A review and evaluation of antibiotic alternatives in the literature. ASAS-ADSA Sectional Meeting, Omaha, NE. March 13-15, Abstract #305

N.K. Gabler, S.M. Curry, **W.P. Schweer**, S.M. Lonergan, K. Schwartz, E.R. Burrough, and C. Loving (2017) What are the nutritional and metabolic costs of immune system activation in pigs? ASAS-ADSA Sectional Meeting, Omaha, NE. March 13-15, Abstract #143

N.R. Gordon, S.M. Curry, **W.P. Schweer**, N.K. Gabler, J.D. Colpoys (2017) Effects of nutrient supplementation on pig sickness behavior when infected with Porcine Reproductive and Respiratory Syndrome Virus. ASAS-ADSA Midwest Sectional Meeting, Omaha, NE. March 13-15, Abstract #405

Gabler, N.K. and **W. Schweer**. Impact of nutrition on disease susceptibility and the impact of disease on nutrient utilization. Pig Feed Quality Conference 2016. March 31-April 2, Bangkok, Thailand.

A.L. O'Connell, **W.P. Schweer**, K. Schwartz, G. Gourley, M.A. FitzSimmons, N. Gabler. (2016) Increased dietary soybean meal does not affect performance during a PRRSV-*Mycoplasma hyopneumoniae* challenge. ASAS-ADSA Midwest Sectional Meeting, Des Moines, IA. March 14-16, Abstract #411.

S.M. Lonergan, E. Huff Lonergan, K. Schwartz, **W. Schweer**, N. Gabler (2015) Viral challenges augment skeletal muscle proteolysis in growing pigs. International Congress of Meat Science and Technology, Clermont-Ferrand, France, August 23-28.

Other Publications

K. Olsen, N. Gabler, **W. Schweer**, K. Schwartz, C. Rademacher, and J. Patience (2017, August) "Evaluating alternatives for antibiotics in nursery pig diets" *National Hog Farmer*

W. Schweer, and N. Gabler (2017, March) "Evaluation of sub-therapeutic growth promotion antibiotic alternatives" *National Hog Farmer*