Examining the effect of non-essential amino acid nitrogen content on lysine requirement for nitrogen retention and growth performance in growing pigs

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ABSTRACT

Low protein (**LP**) diets with crystalline amino acid (**CAA**) supplementation have improved nitrogen (**N**) utilization while maintaining performance in growing pigs. Although LP diets are beneficial, non-essential amino acids (**NEAA**) or N may become limiting in LP diets, affecting N utilization for N retention (**NR**). Consequently, the essential amino acid-nitrogen to total nitrogen ratio (**EAA-N:TN**) may be useful in determining the appropriate amount of EAA and NEAA, or N, that should be provided in LP diets. Therefore, this thesis evaluated the effects of providing a low (**LR**) or high (**HR**) EAA-N:TN ratio on lysine (**Lys**) requirement for NR and growth performance in pigs. An N balance study estimated 1.21% standardized ileal digestible (**SID**) Lys requirement to maximize NR in pigs fed the HR diets. No breakpoint was achieved for pigs fed the LR diets. Therefore, NEAA or N become limiting in HR diets, whereas Lys is limiting in LR diets. A follow-up growth performance study was conducted using the same LR and HR ratios in the diets, with Lys included at 100% NRC requirements, and at the breakpoint value from the N-balance study, formulated for 20-50 kg pigs. Average daily gain (**ADG**), average daily feed intake (**ADFI**), gain:feed (**G:F**), and N output were measured in the growth performance study. Increasing Lys resulted in increased overall ADG and ADFI, but there was no effect of ratio or interactive effect of ratio and Lys. However, pigs fed the HR diets had improved ADG in week 4, whereas pigs fed LR diets had improved ADG and G:F in week 1. In addition, pigs fed the HR diets with 1.22% SID Lys had lower N output compared to pigs fed the other diets. It was concluded that insufficient dietary N may be more of a concern during periods of general nutrient insufficiency, and that diets deficient in dietary N may require more Lys than what is currently recommended. Overall, an increase in Lys may be required to maximize NR and growth, and that NEAA and N become limiting in HR diets. By incorporating the EAA-N:TN ratio in current diet formulations, along with appropriate dietary inclusion of EAA may result in decreased dietary costs for producers and decreased N excretion into the environment, while improving NR and N utilization in growing pigs.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
AIA	Acid insoluble ash
AID	Apparent ileal digestible
Ala	Alanine
AOAC	Association of analytical chemists
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
ATP	Adenosine triphosphate
ATTD	Apparent total tract digestibility
AUP	Animal use protocol
BCAA	Branched chain amino acid
BUN	Blood urea nitrogen
BW	Bodyweight
^{o}C	Degree Celsius
Ca	Calcium
CAA	Crystalline amino acids
CCAC	Canadian council of animal care
CEAA	Conditionally essential amino acids
CP	Crude protein

Cys	Cysteine
d	Day
DAAO	Direct amino acid oxidation
dL	Deciliter
DM	Dry matter
EAA	Essential amino acid
EAA-N	Essential amino acid-nitrogen
EAA-N:TN	Essential amino acid-nitrogen:total nitrogen
g	Gram
GDH	Glutamate dehydrogenase
G:F	Gain:feed
GIT	Gastrointestinal tract
Gln	Glutamine
Gln-S	Glutamine synthetase
Glu	Glutamate
Gly	Glycine
h	Hour
HCl	Hydrochloric acid
His	Histidine
HP	High protein
HR	High ratio
IAAO	Indicator amino acid oxidation
Ile	Isoleucine

kcal	Kilocalorie
kg	Kilogram
Leu	Leucine
LP	Low protein
LR	Low ratio
Lys	Lysine
ME	Metabolizable energy
Met	Methionine
mg	Milligram
min	Minute
N	Nitrogen
NE	Net energy
NEAA	Non-essential amino acid
NEAA-N	Non-essential amino acid nitrogen
NPN	Non-protein nitrogen
NR	Nitrogen retention
NRC	National Research Council
P	Phosphorus
PD	Protein deposition
pH	Power of hydrogen
Phe	Phenylalanine
PROC NLIN	Procedure nonlinear
Pro	Proline

PUN	Plasma urea nitrogen
PWD	Post-weaning diarrhea
R^2	Coefficient of determination
SAS	Statistical analysis software
SEM	Standard error of the mean
Ser	Serine
SI	Small intestine
SID	Standardized ileal digestible
T-AA	Total amino acids
TCA	Tricarboxylic acid cycle
TEAA	Total essential amino acids
Thr	Threonine
TID	True ileal digestible
TiO_2	Titanium dioxide
TN	Total nitrogen
TNEAA	Total non-essential amino acids
Trp	Tryptophan
Tyr	Tyrosine
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
Val	Valine

1.0 GENERAL INTRODUCTION

High protein (**HP**) diets provide the necessary essential amino acid (**EAA**) requirements for the pig but are costly for producers as well as the animal due to the supply of excess protein, and therefore amino acids (**AA**; Wang et al., 2018). The utilization efficiency of nitrogen (**N**) is reduced because the animal uses energy to break down the excess supply of AA and to excrete excess N. Low protein (**LP**) diets along with supplementation of EAA have been introduced to lower the cost of the diet and to improve the animal's utilization efficiency of N, in turn lowering N excretion (Gloaguen et al., 2014). However, LP diets assume that pigs are capable of synthesizing enough non-essential amino acids (**NEAA**) for maximum growth. The synthesis of NEAA requires N, which may not be adequately provided in LP diets (Gloaguen et al., 2014). It is possible that NEAA or total nitrogen (**TN**) become limiting if the crude protein (**CP**) level in the diet is reduced by more than 4% units from 17.6% CP (Gloaguen et al., 2014; Mansilla et al., 2017a). Even though a diet may have excess EAA, the N obtained from their catabolism would be used inefficiently in the synthesis of NEAA (Lenis et al., 1999). This inefficiency is a result of the animal using energy for the catabolism of EAA to obtain N for protein synthesis. If there is a proper proportion of EAA, rather than an excess, to provide an adequate amount of N for protein synthesis, there would be a lower energetic cost to the animal, improved N retention (**NR**), and less N excretion. As a result, it is important to consider NEAA and TN requirements when formulating LP diets supplemented with EAA.

The ideal protein concept has been utilized to estimate the proper balance of AA in order to meet the requirements of growing pigs (Heger et al., 1998). Amino acids have been balanced in accordance with the first limiting AA, lysine (**Lys**), indicating that any AA supplied in excess relative to Lys will not be utilized but rather excreted in urine and feces (Lemme, 2003). If AA are balanced properly (i.e., not supplied in excess) N utilization will be improved. However, the ideal protein concept focuses mainly on EAA, with little consideration given to NEAA requirements. There is increasing evidence that EAA supplementation is not enough for the animal to achieve its maximum growth potential. As a result, more attention has been given to the optimum ratio between EAA-N and TN (**EAA-N:TN**) in order to ameliorate EAA and NEAA and N estimates. Heger et al. (1998) demonstrated that there should be a minimum amount of NEAA supplemented in LP diets in order to improve NR. Through the use of the

EAA-N:TN ratio, these studies will investigate the effects of N deficiency on EAA requirements, with the goal of improving growth performance and NR in the growing pig.

2.0 LITERATURE REVIEW

2.1 Introduction

Amino acids (**AA**) are required by pigs to meet their physiological needs for maintenance and growth. They act as building blocks for proteins and substrates for the synthesis of numerous substances, such as glutathione and polyamines, that are important for various metabolic functions in the animal (Wu et al., 2014). Amino acids are classified as either essential (**EAA**) or non-essential (**NEAA**) and are obtained from the diet or, in the case of NEAA, also produced endogenously. Consequently, diets must contain a source of protein in order to meet the pigs' requirement for EAA, while nitrogen (**N**) must be available to meet the requirements for endogenous synthesis of NEAA. To ensure an adequate supply of EAA, high protein (**HP**) diets were generally fed to pigs in the past, however, these diets often provide more than what the pig requires, resulting in excess protein (Wang et al., 2018). Protein supplied in excess of requirements cannot be utilized for growth and must be eliminated, resulting in an increase in N excretion into the environment and reduction in growth performance (Wang et al., 2018). Gut health of the pig may also be affected by dietary protein supply, due to fermentation of excess protein by microbes in the gastrointestinal tract (**GIT**; Columbus, 2012a). In order to counteract the negative impacts of HP diets, diets are now formulated with reduced or low protein (**LP**), with supplementation of EAA to meet requirements. This minimizes the oversupply of protein while maintaining AA availability and utilization efficiency. Low protein diets have also been shown to be beneficial to gut health, reducing microbial fermentation of protein and production of harmful metabolites (Nyachoti et al., 2006). However, reducing dietary protein level by more than 4% units may have adverse effects on growth performance, even when these diets are supplemented with appropriate amounts of EAA (Gloaguen et al., 2014). Furthermore, NEAA or total nitrogen (**TN**) may be the next limiting factors for growth performance in LP diets (Mansilla et al., 2017a).

Requirements for AA are guided by the ideal protein concept, which assumes an ideal ratio, usually to lysine (**Lys**), of AA supply for maximum growth performance (Heger, 2003). A limitation of the ideal protein concept is its focus on EAA requirements, which have been extensively studied. However, NEAA requirements have been given little regard, even though they supply almost half of the total dietary N required by pigs (Heger, 2003). While it is generally thought that the pig is able to endogenously produce sufficient NEAA to meet requirements, a source of N is required in order for this production to occur. Although N can be supplied from a variety of both protein and non-protein sources, in LP diets the source of this N may be EAA, reducing their utilization for protein synthesis. Consequently, the relationship between EAA and NEAA, expressed on an N basis as the ratio of EAA-nitrogen (**EAA-N**) to TN may be useful in determining an adequate supply of both EAA and N (Heger, 2003).

This literature review focuses on the importance of providing dietary protein with regards to its environmental impact and its effect on growth performance in pigs. In addition, the influence of the ideal protein concept, AA and N metabolism, and the EAA-N:TN ratio on EAA, NEAA, and N requirements for whole-body protein deposition in the growing pig will be discussed.

2.2 Importance of protein in swine

Proteins are involved in many physiological roles, such as acting as catalysts, cell signalling, hormone secretion, gene expression, and gut health (Wu, 2009). Amino acids act as substrates for protein synthesis and are, therefore, involved in muscle protein deposition (Liu et al., 2015). Dietary protein is required to provide AA for use by the animal and an imbalance in the provision of AA has negative effects on feed intake, behaviour, and growth (Wu, 2009). Consequently, knowledge of AA requirements and providing appropriate amounts of protein and, therefore, AA in the diet is essential to maintain optimal growth performance. This section of the review will focus on the environmental impact of dietary protein and the effect of protein on growth performance, with emphasis on the differences between feeding an HP or LP diet.

2.2.1 Provision of protein for optimal growth

High protein diets provide sufficient Lys to meet the pigs' requirements for growth. It is assumed that by providing growing pigs with HP diets that all EAA requirements, in addition to Lys, are being met and that the animal can utilize the protein provided for the sufficient production of endogenous NEAA for growth. Although these diets have been adequate in pig production in the past, HP diets provide an excess amount of protein and AA that has a negative impact on pig performance and the environment. Low protein diets supplemented with crystalline amino acids (CAA) were introduced to improve nutrient utilization efficiency and, therefore, performance (Wang et al., 2018).

Decreasing dietary crude protein (CP) content up to 8% units below suggested requirements given by the National Research Council (NRC, 2012) provide pigs with similar growth performance to those on regular HP diets when the LP diets were supplemented with AA (Morales et al., 2015). For instance, Buraczewska and colleagues (2006) determined that 25-50 kg pigs fed an LP diet containing, 16% CP and supplemented with Lys and threonine (Thr) had improved N retention (NR) and growth performance than pigs fed an 18% CP diet supplemented with methionine (Met). Furthermore, the addition of glycine (Gly) and arginine (Arg) to an LP diet of 13% CP supplemented with Lys, Met, Thr, tryptophan (Trp), isoleucine (Ile), and valine (Val) resulted in similar growth performance in 20-50 kg pigs than an 18% CP diet (Powell et al., 2011). However, NR and growth performance of pigs are negatively impacted when dietary CP is reduced by more than 4% units, from a diet with 17% CP, even with provision of EAA and is indicative of N being limiting in the diet (Otto et al., 2003; Guay et al., 2006; Columbus, 2012; Gloaguen et al., 2014). Similarly, Millet et al. (2018a) suggested that N, or CP, was limiting growth at low CP levels. The addition of glutamate (Glu), Gly, proline (Pro), and serine (Ser) to protein-free diets improved NR (Křížová et al., 2001). For example, Glu and glutamine (Gln) are required for optimal growth, intestinal health, and production in pigs (Wu et al., 2011; Rezaei et al., 2013). Evidently, the proper amounts of AA and N need to be taken into consideration for optimal growth performance.

During the weaning period, piglets are subject to a transition phase from liquid to solid feed, including a shift in protein source and content, which has a major impact on their GIT, generally causing post-weaning diarrhea (PWD; Wang et al., 2018). The provision of HP diets has been shown to increase the incidence of PWD by providing excess protein that results in an ideal environment for the growth of pathogenic bacteria (Macfarlane and Gibson, 1995). In addition, the microbial degradation of dietary protein yields numerous metabolites, such as ammonia and branched-chain fatty acids, through the process of fermentation, such as ammonia and branched-chain fatty acids, which may negatively affect the GIT and growth performance of pigs by impacting their N metabolism and AA supply (Jensen, 2001; Blachier et al., 2007; Heo et al., 2008; Columbus, 2012). Reducing dietary protein content has been shown to reduce microbial activity, improving AA supply to the host (Columbus, 2012).

2.2.2 Environmental impact of dietary protein

Traditional corn-soybean meal-based diets can provide an excess amount of protein to meet the pigs' requirements for AA. While meeting requirements, HP diets oversupply protein and AA, with the excess catabolized and excreted into the environment as N. This represents a major environmental impact of the pork industry; however, strategies are available to reduce the excretion of N from pork production (Leip et al., 2014; Wang et al., 2018). For example, inclusion of fermentable fibre in diets results in a shifting of the route of N excretion from urine to feces via the incorporation of urea-N into microbial protein to slow the breakdown of protein (Aarnink and Verstegen, 2007). Phase feeding has been shown to decrease N excretion by more closely matching dietary supply of AA to the pig's requirements for AA during a particular life stage (Millet et al., 2018b). For instance, Remus et al. (2018) determined that finishing pigs fed using a precision feeding system consumed less protein and had 17% less N excretion and improved NR than those in conventional group housing. One of the most beneficial strategies for reducing N excretion is the reduction of protein content in diets. The increase in availability of synthetic AA has allowed for the reduction of dietary protein supply, while maintaining dietary EAA to meet requirements, reducing the oversupply of protein. Reducing the dietary protein supply by 10 g/kg has been shown to decrease ammonia emissions by up to 10% (Wang et al., 2018).

2.3 The ideal protein concept

The ideal protein concept was developed to get a better understanding of an optimum balance of AA in order to meet requirements for maintenance and growth (Heger et al., 1998). In past years, and currently, the focus has been on EAA requirements for protein deposition and growth in pigs (Wu et al., 2014). Although important, EAA only supply about half of the total N in the diet (Heger et al., 1998). The remaining half presumably comes from NEAA or other non-protein nitrogen (NPN) sources (Heger et al., 1998). Consequently, it is important to consider incorporating NEAA requirements into the concept of ideal protein to achieve the proper balance between EAA and NEAA, facilitate maximum protein utilization or growth, and to reduce the amount of N excreted (Stucki & Harper, 1962; Heger et al., 2003). In addition, AA content of the diet does not necessarily reflect the composition of AA in the pig due to first-pass metabolism, with many AA being catabolized in the small intestine (SI), splanchnic tissues, and liver (Wu et al., 2014). This section of the review focuses on the foundational understanding of EAA and NEAA through their classification, the determination of their requirements, and current issues with feeding very low protein diets.

2.3.1 Classification of amino acids

Essential AA are defined as those AA that must be provided in the animal's diet, as they cannot be synthesized endogenously. Essential AA for swine include Met, Lys, Trp, Thr, phenylalanine (Phe), Ile, Val, leucine (Leu), and histidine (His). Non-essential amino acids are those that the animal can synthesize *de novo,* and, therefore, do not need to be supplemented in the diet. However, there has not been concrete evidence to support the theory that NEAA are sufficiently synthesized by pigs, or any monogastric mammal, to support their physiological functions (Reeds, 2000; Wu 2010). In addition to these major classifications, some AA are classified as semi-essential, or conditionally essential (CEAA). Conditionally EAA are those that can be synthesized in adequate amounts under normal conditions, but whose synthesis may not be sufficient when their utilization is greater, such as during gestation, lactation, weaning, and stress (Wu, 2009; Wu et al., 2014). Amino acid synthesis is dependent upon numerous factors, such as species, age, and intestinal microbiota (Wu, 2013). Consequently, classifying AA as EAA or NEAA is an intricate process, indicating the necessity of proper classification to meet requirements for all AA.

2.3.2 Determining amino acid requirements

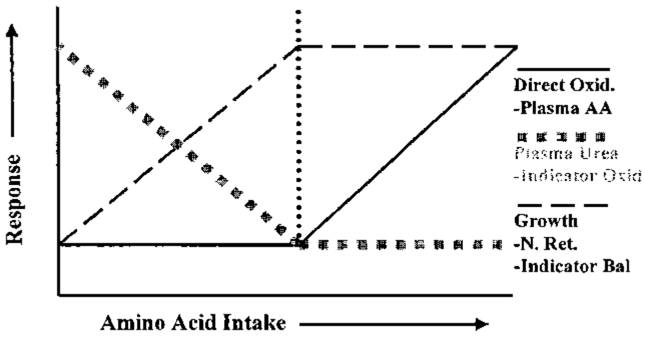

Figure 1. The three different patterns of metabolic responses to graded intakes of an essential amino acid (Figure 1, Pencharz and Ball, 2003)

Current methods used to determine AA requirements involve feeding various levels of a test AA (usually an EAA) to the animal while measuring a biological change in response to the increasing supply (Pencharz and Ball, 2003). The point at which the response changes, either reaching a plateau or increasing depending on the response, indicates the requirement. The NRC (2012) provides swine nutritionists with guidelines for determining AA requirements. For instance, the test AA should be deficient in the diet being used, and all other nutrients should meet requirements. In addition, there should be at least 4 graded levels of the test AA. Finally, an appropriate duration and statistical model for the experiment are required based on the response being evaluated. Methods to determine AA requirements are primarily based on measuring protein deposition, directly or indirectly, and involve responses such as N-balance and growth, plasma AA level, and plasma urea concentrations (Pencharz and Ball, 2003, Figure 1). These parameters are used to determine the response to the test AA given at different levels in the diet. Nitrogen balance and growth are the most common outputs used to determine EAA requirements in growing animals. Using these outputs, N-balance for whole body or for a specific tissue may be determined (Wu et al., 2014). Although effective, N-balance does not account for all N losses from a subject, affecting the estimation of AA requirements (Wu et al., 2014). Nitrogen-balance tends to underestimate the requirement, as a result of overestimating N intake and underestimating N excretion (Elango et al., 2012). These outputs also disregard AA requirements for other physiological functions, such as those related to the health of the animal. The direct AA oxidation (DAAO) method involves measurement of the catabolism of the AA whose requirements are being determined (Pencharz and Ball, 2003). The indicator AA oxidation (IAAO) method is used to measure the requirement of an AA through the use of an indicator AA (Elango et al., 2012). The oxidation of the indicator AA is measured at increasing intakes of the limiting AA, and requirement of the limiting AA is defined once no more changes are observed in the oxidation of the indicator AA (Elango et al., 2012). Through the use of IAAO, it is possible to determine whether the AA is incorporated into protein, a secondary metabolite (for instance, Phe being utilized to produce fumarate), or oxidized (Pencharz and Ball, 2003).

In using the factorial method, dietary requirements for an AA for use by the whole body or specific tissue may be determined (Wu et al., 2014). The factorial method involves feeding a protein-free diet and measuring the changes in the sum of faecal and urinary N, AA deposition, and AA excretion in response to the protein-free diet (Wu et al., 2014). When it is unknown at which rates AA are used in the body or tissues, the factorial method can only be used when information on AA oxidation in the test subject's body is known (Wu et al., 2014). Methods used to determine AA requirements are largely focused on EAA requirements, because determination of NEAA is proven to be more difficult, and due to the belief that NEAA do not need to be provided in the diet. However, NEAA requirements should be considered when formulating diets as they may not be synthesized sufficiently by the animal. For instance, Wu (2014) mentioned that although cysteine (Cys) and tyrosine (Tyr), both considered NEAA, can be synthesized from Met and Phe respectively, there is no *de novo* synthesis of Cys and Tyr because their precursors are not present in the animal unless provided in the diet. Cysteine and Tyr have important functions in intestinal cells and without adequate supplementation of Met and Phe, there may not be enough Cys and Tyr production for the pig to function properly (Wu, 2014). Current estimates of requirements are based on experiments in which pigs were fed HP diets, and methods may need to be re-evaluated to include estimates for requirements when feeding LP diets. Specifically, requirements for NEAA, N, and the roles of AA for physiological functions other than NR and growth performance, to improve productivity and the cost of feed.

2.3.3 Use of the ideal protein concept

The ideal protein concept has provided nutritionists with the foundational information that may be used to formulate diets to appropriately meet requirements. For instance, AA requirements expressed relative to Lys is still very useful in current diet formulation (van Milgen and Dourmand, 2015). This concept, in addition to the increased availability of CAA, has led nutritionists to formulate LP diets with EAA supplementation (van Milgen and Dourmand, 2015). Although this has been effective since first proposed for swine in the 1980s, the ideal protein concept should be re-evaluated as it mainly describes the ideal amount of EAA that should be supplied to meet requirements, and ignores NEAA (Wu and Li, 2022). Formulating diets with the basis that the EAA from the diet exactly match the animal’s requirements is flawed for various reasons. For instance, AA coming from the diet undergo different rates of catabolism, and their concentrations in the portal-drained viscera are different than what would be present in the diet (Wu and Li, 2022). Similarly, the metabolism of AA varies across different protein sources (i.e., in general, plant protein is less digestible than animal protein). Amino acid requirements vary at different stages, and the ideal protein concept was described as a general guideline. For instance, the AA composition of weaning pigs differ from that of grower pigs (Wu and Li, 2022).

Although there have been numerous studies on the positive effects of the addition of CAA to LP diets, results indicate that N may be limiting optimal production performance and NEAA or N should be added to diets (Heger et al., 1998; Křížová et al., 2001; Mansilla, 2013; Peng et al., 2016; Mansilla et al., 2017a; Hofmann et al., 2020). Due to increased availability of CAA, the ideal protein concept should be redefined to incorporate EAA, NEAA, and N requirements for various stages of growth and consider the roles of AA for physiological functions in order to optimize overall pig performance.

2.4 Amino acid and nitrogen metabolism

Although dietary protein supplies the growing pig with AA and N required for protein deposition and growth, not all those AA and N are available to the animal. Consequently, it is important to understand the difference between what is supplied and digested versus what the pig can utilize (i.e., bioavailability). This section of the review will focus on AA and N metabolism in the growing pig and important considerations regarding bioavailability and utilization efficiency when formulating diets to improve growth performance and swine production.

2.4.1 Amino acid bioavailability

Measuring the bioavailability of AA is essential to provide appropriate amounts of AA to the growing pig to reduce the inefficient utilization of excess AA. The bioavailability of AA represents the amount of AA that are digested in a chemical form readily available for metabolism or protein synthesis (Batterham, 1992). Slope-ratio assays have been used to determine the bioavailability of a particular AA, where an ingredient is fed to supply increasing, but limiting, levels of the test AA (Batterham, 1992). The slope of the response to the test ingredient is compared to the slope of a diet assumed to have 100% AA bioavailability, with this ratio representing the bioavailability of the test AA (Stein et al., 2007). These assays tend to underestimate bioavailability and are costly as only one AA can be tested at one time (Stein et al., 2007). Consequently, AA digestibility has been accepted as a more suitable proxy measure of AA bioavailability. Specifically, ileal digestibility, rather than total tract digestibility, is used to estimate AA availability as AA are largely absorbed intact from the SI, with AA disappearance from the hindgut being confounded with microbial fermentation and AA production (Sauer and Ozimek, 1986; Stein et al., 2007; Columbus and de Lange, 2012). There are three measurements of digestibility that represent the disappearance of AA from the GIT at the ileum; apparent ileal digestibility (AID), standardized ileal digestibility (SID), and true ileal digestibility (TID; Stein et al., 2007).

Apparent ileal digestibility represents the disappearance of AA from the GIT at the terminal ileum, without consideration of the endogenous AA losses (Stein et al., 2007). Standardized ileal digestibility measurements consider basal endogenous AA losses and represent a more accurate estimate of AA bioavailability than AID values (Stein et al., 2007). Finally, TID accounts for total AA losses, endogenous and feedstuff-specific, although there is no method currently available to determine feedstuff-specific losses (Stein et al., 2007).

2.4.2 Amino acid metabolism

The enzymatic digestion of AA in non-ruminants begins in the upper gut, specifically the stomach, where proteins are denatured to facilitate enzymatic digestion in the SI (Krehbiel and Matthews, 2003). The end products of AA digestion are free AA and di- and tripeptides (Krehbiel and Matthews, 2003). These AA and peptides have various fates, such as incorporation into proteins, metabolized by the SI for energy, or entering the hepatic portal circulation for whole-body use (Krehbiel and Matthews, 2003). The pig's AA requirements for maintenance and growth change depending on the life stage. For instance, during the growth stage, the pig is partitioning ingested nutrients towards maintenance and growth. Consequently, the pig will require adequate AA for basic functions such as protein turnover and immune function, with additional AA to satisfy growth requirements. In contrast, during maturity, the pig will utilize the majority of ingested nutrients for maintenance functions. Glutamine, Glu, Arg, Thr, and Cys are heavily involved in maintenance functions, specifically those related to immunity and gut health (Bequette, 2003). Glutamine is required as an energy source for the SI and for the synthesis of other NEAA. During growth, weaning, and stress, its supply may be limited due to an increase in turnover and more Gln may be required to maintain regular bodily functions and growth (Wu et al., 1996; Bequette, 2003). The majority of dietary NEAA undergo extensive oxidation in the SI by enterocytes during first-pass metabolism, with low concentrations entering the portal vein (Stoll et al., 1998; Wu 1998). In addition, 30-50% of dietary EAA are catabolized by the SI during first-pass metabolism (Stoll et al., 1998; Wu 1998). As a result, it is important to consider AA metabolism to understand when requirements differ and how to compensate for different lifestages when formulating diets.

Amino acids that are not utilized for protein synthesis are broken down into an amino group and a carbon skeleton. Carbon skeletons from the catabolism of AA result in the formation of glucose and/or ketones. The amino group is used to generate other AA, ammonia, or urea, and the carbon skeleton is used for energy by various tissues in the body. Transamination reactions are the initial step in the catabolism of AA, and result in the production of NEAA from a donor AA (Mansilla, 2013). Transamination reactions may be coupled with the action of the enzyme glutamate dehydrogenase (GDH) in the liver, which results in the production of ammonia (D’Mello, 2003). Ammonia is then reutilized to form Gln or converted to urea to be excreted via the kidneys (D’Mello, 2003). Branched-chain AA (BCAA) are transaminated in skeletal muscle, where their respective amino groups are used to produce Gln, and their carbon skeletons are transported to the liver to be metabolized for energy (Cemin et al., 2019).

Following the catabolism of AA, the amino group is released as ammonia, which can be reincorporated into Gln or converted to urea (D’Mello, 2003). Glutamine acts as an inter-organ N transporter, enabling various tissues to synthesize NEAA when they are required (Young and Ajami, 2001). Ammonia that cannot be incorporated into Gln is incorporated into urea, preventing a buildup of ammonia and ammonia toxicity (D’Mello, 2003). In the liver, ammonia enters the urea cycle as Asp or ammonium, undergoing various reactions to produce urea to be excreted in urine.

2.4.3 Synthesis of NEAA

The metabolic process of NEAA synthesis contributes to the efficiency of use of compounds supplied by the diet. It is essential to understand this process to formulate diets with adequate AA to meet requirements for efficient growth. The synthesis of NEAA is dependent on the dietary supply of EAA and N, and although there may be enough EAA to meet requirements, the synthesis of NEAA may still be insufficient (Wu et al., 2010). Non-essential AA synthesis occurs through transamination reactions or directly from EAA, for instance the production of Cys from Met. Transamination reactions involve the transfer of an amino group from a donor AA, such as Glu, to an α-keto-acid. Similar to urea, NEAA are synthesized in the liver with Glu as a precursor (Bequette, 2003). Glutamate may be added in the diet, but it is mainly synthesized *de novo* from NPN sources, such as ammonia or urea, and carbon skeletons obtained from intermediates of glycolysis and the tricarboxylic acid cycle (TCA) such as 3-phosphoglycerate, pyruvate, oxaloacetate, and α-ketoglutarate (Bequette, 2003). Glutamate dehydrogenase is the enzyme responsible for the formation of Glu using ammonia and α -ketoglutarate (Mansilla, 2013). The remainder of the NEAA, other than Gln, Pro, and Gly, are formed via transamination reactions and obtain their amino groups or carbon skeletons from other AA (Bequette, 2003, Mansilla, 2013). The formation of Gln is catalyzed by glutamine synthetase (Gln-S) and involves the addition of ammonia to Glu via adenosine tri-phosphate (ATP) phosphorylation (Berg et al., 2002). Proline and Arg are derived from the reaction of ATP with Glu’s carboxyl group, producing the precursor glutamic semialdehyde (Berg et al., 2002). This precursor may be used to form Pro and Arg (Berg et al., 2002). Serine is formed from 3-phosphoglycerate through transamination reactions and can be used to produce Gly and Cys (which also requires a sulfur atom from Met; Berg et al., 2002). Glutamate acts as an amino group donor for the formation of aspartate (Asp) and alanine (Ala) using oxaloacetate and pyruvate, respectively (Berg, et al. 2002). Asparagine (Asn) is synthesized from Asp in a process similar to that of obtaining Gln from Glu. Tyrosine is considered an NEAA but requires Phe to be synthesized.

Under the current ideal protein concept, it is presumed that sufficient NEAA are being provided in the diet and/or synthesized endogenously in adequate amounts. However, as previously stated, NEAA availability and N are reduced in LP diets. Consequently, the synthesis of many NEAA may be insufficient to meet requirements, specifically that of CEAA, impacting the animal’s growth performance. Overall, with less N available to the animal, the synthesis and utilization efficiency of NEAA will be negatively impacted.

2.4.4 Importance of meeting NEAA requirements

The supply of AA in current diets, especially LP diets, may prevent sufficient endogenous synthesis of NEAA, reducing maximal protein deposition in skeletal muscle and efficient utilization of EAA in growing pigs (Wu et al., 2014). Catabolism of excess EAA may occur in order for the animal to obtain the N required for NEAA synthesis, resulting in inefficient utilization of EAA. In the case of LP diets, non-essential N may become limiting, preventing the animal from achieving their maximum growth potential (Wang et al., 2018). In a study by Deng et al. (2009), newly weaned piglets were unable to adequately synthesize protein in extra-intestinal tissues as a result of reduced dietary NEAA content, limiting their growth performance when fed diets containing 12.7% CP and supplemented with sufficient dietary EAA. It was also concluded that a lack of NEAA may prevent the efficient re-utilization of EAA obtained from the breakdown of body protein, due to the use of EAA to produce NEAA rather than be used for other functions (Křížová et al., 2001). In support of this hypothesis, an N-balance study by Křížová et al. (2001) observed that the addition of NEAA to protein-free diets improved NR and reduced N loss. Similarly, Hofmann et al. (2020) investigated the effects of adding combinations of the NEAA Asp, Asn, Gln, and Glu to 160 g/kg CP diets in chickens to match the NEAA concentrations of a CP diet containing 180 g/kg. They concluded that these NEAA aided in reverting the negative growth effects of LP diets. Glutamine is required as an energy source for the SI and for the synthesis of NEAA. During growth, weaning, and stress, its supply may be limited due to an increase in turnover and more Gln may be required to maintain regular bodily functions and growth (Wu et al., 1996; Bequette, 2003). Overall, the addition of

NEAA to LP diets may improve N utilization and NR, improving overall growth performance in pigs.

2.5 The use of low protein, amino acid supplemented diets

Low protein diets have become increasingly common due to the demands and costs of protein sources and as a means to improve the environmental impact of pork production. These diets have proven to be cost-effective, while improving NR in pigs, and lowering N excretion into the environment (Wang et al., 2018). In addition, feeding LP diets to weaning pigs, specifically a 2% unit reduction in CP relative to NRC (2012) recommendations, may improve the incidence of PWD due to the decreased amount of undigested protein available for fermentation in the hindgut (Nyachoti et al., 2006; Wang et al., 2018). Furthermore, the inclusion of CAA to LP diets may have a positive impact on pig production by modifying their immune status, promoting intestinal and skeletal growth, and aiding gut health (Wang et al., 2010; van der Meer et al., 2016). Although there are many benefits of LP, CAA supplemented diets, there are still some areas of research that need to be addressed, particularly in relation to growth performance.

2.5.1 Issues with feeding low protein diets

Although LP diets are cost effective, reduce N output from swine production, and benefit gut health of pigs, there are conflicting results in regards to overall growth performance. For instance, growth performance may be maintained when dietary CP content is reduced by up to 8% units if all EAA are supplemented, however, reducing dietary CP by up to 3% units below NRC (1998) recommendations, with the addition of some CAA, not including BCAA, resulted in decreased growth performance in grow-finish pigs (Prandini et al., 2013; Morales et al., 2015). Similarly, there was decreased growth performance in grow-finish pigs when CP was reduced by 4.8% units, even with CAA supplementation (Roux et al., 2011). This reduction was alleviated with the addition of BCAA, implying that all EAA should be added to LP diets when dietary CP content is decreased by more than 3% units (Roux et al., 2011). A further reduction in CP content by 8.3% units, resulted in decreased growth performance, regardless of sufficient EAA supplementation (Guay et al., 2006). At this point, it is suggested that NEAA or N may be limiting in very low protein diets (Mansilla et al., 2017a). For instance, it was demonstrated that the addition of NEAA to protein-free diets improved growth performance (Křížová et al., 2001). Consequently, more research is required to determine requirements for NEAA and N when pigs are being fed very low protein diets.

2.6 The essential to non-essential amino acid ratio

Nitrogen utilization is defined by how well the animal is able to use N obtained from the diet for specific functions, such as its incorporation into AA for growth. In this section the ratio of EAA-N:TN and its impact on N utilization and efficiency will be reviewed.

2.6.1 Estimating the optimum essential to non-essential amino acid ratio

Due to the increasing prevalence of diets formulated with reduced protein and EAA supplementation, an estimation of the adequacy of these diets to meet both EAA and NEAA requirements of the pig is necessary. Nitrogen is required for endogenous NEAA synthesis and may become a limiting factor for the animal in LP diets (Heger, 2003). The EAA-N:TN ratio has been investigated by various researchers to identify the proper quantity of EAA and NEAA that should be supplied in LP diets (Wang and Fuller, 1989; Lenis et al., 1996; Heger et al., 1998). However, defining this ratio is difficult as the requirements for NEAA and N are not well defined. In addition, the various opinions on the classification of certain AA as CEAA adds difficulty in determining the appropriate ratio, as this would change which AA are included in each fraction, resulting in different optimal EAA-N:TN ratios for different lifestages and circumstances.

There are two distinct methods researchers may use to determine the optimum EAA-N:TN ratio, resulting in different estimates of optimal EAA-N:TN ratio. The first involves keeping the concentration of total dietary N constant, while measuring the changes in EAA-N and non-essential amino acid N (NEAA-N) (Heger, 2003). The second method measures the amount of TN that is required for physiological responses, by keeping the concentration of EAA-N constant (Heger, 2003). By utilizing the first method, it is important to consider the effects of choosing either a high or low TN concentration as that will influence the EAA-N estimate, which will further impact NR. For instance, a higher TN value will result in a lower EAA-N requirement, and therefore a lower ratio as optimal (Heger et al., 1998). The second method seems to be the more appropriate method as the TN fraction may be altered while EAA-N remains constant, providing estimates of the amount of TN that is required to achieve an optimal ratio for NR (Heger, 2003). Overall, it is important to determine a proper balance between EAA-N and NEAA-N to meet the animal's requirements for maximum growth.

Many values for the EAA-N:TN ratio have been determined across multiple species. Stucki and Harper (1962) conducted a study using EAA-N:TN ratios between 0.33 to 1.0, representing diets with a high amount of NEAA and EAA, respectively, and observed lower performance in rats fed diets with extreme ratios. Rats fed diets only containing EAA also had low growth performance as a result of lower feed intake (Stucki and Harper, 1962). No improvement in performance was observed when EAA-N:TN was between 0.50 and 0.81, indicating the optimum ratio to be around 0.50 (Stucki and Harper, 1962). Similarly, Young and Zamora (1968) observed the effects of EAA-N:TN ratios between 0.14 to 0.93 in growing rats being fed at two dietary CP levels. It was concluded that the optimum ratio decreased as the CP levels increased and that extreme ratios resulted in lower growth performance (Young and Zamora, 1968).

Lenis et al. (1999) studied NR in pigs using three EAA-N:TN ratios with three different dietary N concentrations and observed that the optimum EAA-N:TN ratio to achieve maximum NR and utilization is 0.50. Heger et al. (1998) used a range of six EAA-N:TN ratios from 0.25 to 0.86 and observed a curvilinear response with maximum NR being observed at 0.6 when total N in the diet was kept constant. At a constant level of dietary EAA-N, NR was decreased above a ratio of 0.48 (Heger, 1998).

In a recent study conducted in broiler chickens, Maia et al. (2020) observed the effects of EAA-N:TN ratios ranging from 0.44 to 0.56. Six different diets were used, one control diet with a high CP level of 225 g/kg and low EAA-N:TN ratio of 0.47, one diet with a CP level of 190 g/kg and a ratio of 0.56, and four diets with CP levels of 190 g/kg with a NEAA mixture at varying inclusion levels and ratios of 0.44, 0.47, 0.50, and 0.53. The chickens consuming the diet with a ratio of 0.56 had a lower final body weight and feed conversion ratio than those on the control diet. It was concluded that a ratio of 0.50 resulted in maximum N utilization, although chickens consuming diets with a ratio of 0.50 or less may also be used as they had better feed conversion efficiency when NEAA were added to the diets compared to the higher ratios (Maia et al., 2020).

Optimal EAA-N:TN values are determined by the amount of N present in a diet, however, increasing or decreasing ratios affect NR and N utilization. It has been observed that when EAA-N is held constant, a lower EAA-N:TN ratio was required for maximum NR than for maximum N utilization, implying that protein deposition is maximized until total N becomes limiting (Heger, 2003). Once total N is limiting, protein deposition is decreased, as EAA are then utilized as a source of N for NEAA production. Extreme values of EAA-N:TN ratios are thought to decrease N utilization as a result of insufficient EAA (low ratios) or total N (high ratios) (Heger, 2003). In the latter scenario, it is hypothesized that EAA utilization efficiency is reduced as a result of EAA being utilized to supply N for NEAA production.

2.7 Conclusion

Although LP diets supplemented with EAA are effective in improving growth performance and productivity in growing pigs, the ideal protein concept should be re-evaluated to incorporate NEAA and N requirements. In addition, the EAA-N:TN ratio should be studied further in order to improve the current understanding of EAA requirements, while taking into consideration NEAA and N requirements. Supplying EAA or N in inadequate amounts may decrease productivity and prevent the animal from reaching its genetic growth potential. Consequently, providing LP diets with appropriate amounts of EAA and N may be beneficial to enhance growth performance and reduce the environmental impact of pork production.

3.0 RESEARCH RATIONALE HYPOTHESES AND OBJECTIVES

3.1 Research Rationale

High protein diets have been given to pigs to ensure they are receiving adequate amounts of EAA to maximize growth. However, these diets are providing an excess amount of protein, leading to decreased utilization efficiency and increased N excretion (Wang et al., 2018). Consequently, LP diets with EAA supplementation were introduced and improved the availability of AA to the growing pig and lowered N excretion. Although effective, LP diets with a reduction in CP content by more than 4% units from 17% CP have shown to have negative effects on growth performance even with supplemented EAA, indicating that NEAA or N may be the limiting factors in this case (Gloaguen et al., 2014; Mansilla et al., 2017a). The EAA-N:TN ratio has been introduced to consider the amount of EAA and N the pig is receiving from the diet (Wang & Fuller, 1989; Lenis et al., 1996; Heger et al., 1998). When an excess of EAA is being supplied, or at a high EAA-N:TN ratio, the pig will utilize its energy to catabolize the excess EAA to be excreted or to produce NEAA, rather than use it for growth (Heger, 2003). In addition, if the EAA-N:TN ratio is too low, there is an inadequate supply of EAA (Heger, 2003). Heger et al. (1998) determined that maximum NR was observed when pigs were fed diets with a ratio of 0.48.

Utilization efficiency and production costs may be improved with the application of the EAA-N:TN ratio in growing pig diets as it would ensure EAA and NEAA were being supplied in appropriate proportions to achieve optimal NR and reduce N excretion. Therefore, the purpose of the studies in this thesis was to investigate the effects of a high EAA-N:TN ratio and an optimal EAA-N:TN ratio, as defined by Heger et al. (1998), in LP diets on EAA requirements, NR and growth performance in growing pigs.

3.2 Research Hypotheses

1. A diet deficient in NEAA-N (high ratio) will increase lysine requirement compared to a diet with sufficient NEAA-N (low ratio) for maximum nitrogen retention in growing pigs (Chapter 4).
2. Growth performance of pigs fed diets sufficient in NEAA-N or deficient in NEAA-N but with supplemental lysine would be improved (Chapter 5).

3.3 Research Objectives

1. To determine the effect of the EAA-N:TN ratio on lysine requirement for nitrogen retention (NR) in growing pigs (Chapter 4).
2. To determine the effect of feeding diets with limiting or sufficient non-essential amino acid nitrogen (NEAA-N) with increasing lysine levels on growth performance and N output of growing pigs (Chapter 5).

4.0 THE EFFECT OF THE ESSENTIAL AMINO ACID-NITROGEN TO TOTAL NITROGEN RATIO ON LYSINE REQUIREMENT FOR NITROGEN RETENTION IN GROWING PIGS

4.1 Abstract

Low protein (**LP**) diets supplemented with essential amino acids (**EAA**) fed to growing pigs reduce the supply of excess amino acids (**AA**) and nitrogen (**N**). However, LP diets may become limiting in non-essential amino acids (**NEAA**) and N, thus affecting the utilization of EAA for N retention (**NR**). The ratio of EAA-N:total dietary nitrogen (**TN**) of 0.48 may be required for optimal NR, due to the provision of appropriate quantities of EAA-N and TN. An N-balance study was conducted to determine the lysine (**Lys**) requirement for maximum NR when pigs are fed diets with an optimal or high EAA-N:TN ratio. A total of 80 growing barrows (21.5 kg initial BW; SD = 0.89 kg) were randomly assigned to 1 of 10 dietary treatments ($n = 8$) in 8 blocks in a 2 × 5 factorial arrangement. Diets consisted of a low ratio (**LR**; 16.78% crude protein; EAA-N:TN of 0.48) or a high ratio (**HR**; 15.51% crude protein; EAA-N:TN of 0.55) with graded levels of Lys (0.82%, 0.92%, 1.02%, 1.12%, and 1.22% standardized ileal digestible [**SID**]) fed at 2.8 × maintenance metabolizable energy requirements. After a 7-d dietary adaptation, a 4-d N-balance collection was conducted. Blood samples were obtained on d 2 of the collection period for plasma AA and plasma urea N (**PUN**) analysis. Data were analyzed using PROC MIXED with fixed effects of ratio, Lys, and their interactions and block as a random effect. Lysine requirement was estimated using PROC NLIN breakpoint model. Nitrogen retention increased linearly, and PUN decreased with increasing Lys ($P < 0.01$). Total EAA concentrations were increased in pigs fed HR diets ($P < 0.05$) and decreased with increasing Lys ($P < 0.01$). No effects were observed on total NEAA and total AA concentrations. A significant interaction between ratio and Lys ($P < 0.01$) was observed for NR and total EAA concentration ($P = 0.01$). Quadratic breakpoint model estimated SID Lys required to maximize NR of pigs fed HR diets at 1.22% ($R^2 = 0.53$). A linear response was observed in pigs fed LR diets ($R^2 = 0.80$) with no breakpoint achieved. These results indicate that NEAA or N becomes limiting in HR diets but not in LR diets, and more EAA are required in LR diets to meet requirements for NR. Further research should be conducted utilizing the EAA-N:TN ratio in LP diets to determine an appropriate ratio for optimal Lys utilization.

4.2 Introduction

High protein (**HP**) diets provide the necessary essential amino acids (**EAA**) to meet pigs' requirements but tend to supply an excess amount of protein and, therefore, amino acids (**AA**), reducing the utilization efficiency of nitrogen (**N**) (Wang et al., 2018). Excess protein in the diet results in the animal having to utilize energy to catabolize excess AA rather than use that energy towards growth (Wang et al., 2018). Consequently, low protein (**LP**) diets with supplemental EAA have been introduced to improve the animal's feed efficiency, while reducing N excretion and maintaining growth performance in 10-20 kg pigs (Gloaguen et al., 2014). However, N or non-essential amino acids (**NEAA**) may become limiting in LP diets as these diets supply lower amounts of N to the pig, preventing sufficient endogenous synthesis of NEAA (Mansilla et al., 2017b). In addition, the utilization efficiency of EAA for protein deposition (**PD**) may be reduced as the N from EAA may be used to synthesize NEAA, rather than be used for PD (Wang et al., 2018).

The ideal protein concept focuses on meeting the animal's EAA requirements, with little consideration given to NEAA requirements, even though they supply almost half of the total dietary N required by the pig (Heger, 2003). Consequently, the ratio between EAA-N and total nitrogen (**EAA-N:TN**) has been suggested to depict the relationship between EAA and NEAA on an N basis (Heger, 2003). This ratio considers the appropriate amount of N coming from the diet as EAA and NEAA, improving the EAA, NEAA, and N estimates for N utilization. Heger et al. (1998) demonstrated that there should be a minimum amount of NEAA supplemented in diets in order to improve N retention (**NR**) and concluded that the optimal ratio to maximize NR was 0.48. The objective of the present study was to determine the effect of the EAA-N:TN ratio on lysine (**Lys**) requirement for NR in growing pigs. We hypothesized that the Lys requirement would be increased in pigs fed a diet with a high EAA-N:TN ratio (>0.48).

4.3 Materials and Methods

The experimental protocol was reviewed and approved by the University of Saskatchewan's Animal Research Ethics Board (AUP #20130054) and followed the Canadian Council on Animal Care guidelines (CCAC, 2009).

4.3.1 Animals, Housing, and Diets

A total of 80 growing barrows of 21.5 ± 0.89 kg initial body weight were used in an N-balance experiment at the Prairie Swine Centre, Inc. (Saskatoon, SK). The pigs were housed individually in metabolism crates (1.4 m x 1.5 m) in a temperature-controlled room (22 ± 1 °C). Pigs were randomly assigned to 1 of 10 dietary treatments over 8 blocks in a 2 × 5 factorial arrangement in a randomized complete block design (*n* = 8 pigs/treatment). The factors were dietary EAA-N:TN ratio, with a high ratio of 0.55 (**HR**) and a low ratio of 0.48 (**LR**), and dietary Lys content (0.82, 0.92, 1.02, 1.12, and 1.22% standard ileal digestible [**SID**] Lys). Ratios were formulated to be deficient in dietary N (HR) or to meet the optimal ratio (LR) as determined by Heger et al. (1998). Dietary Lys content was formulated to be 80, 90, 100, 110, and 120% of the requirements according to NRC (2012). Diets were formulated to meet or exceed NRC (2012) requirement for all other nutrients and contained titanium dioxide as an indigestible marker (Table 4.1). The diets containing the lowest and highest levels of Lys were formulated and mixed in appropriate proportions to obtain the 0.92, 1.02, and 1.12% SID Lys diets. Feed intake was provided at 2.8 × maintenance metabolizable energy requirements [$(2.8 \times 110 \times BW^{0.75})$] fed in equal meals twice daily at 0700h and 1500h with ad libitum access to water. Feed refusals were collected for each pig daily and weighed to determine daily feed intake.

4.3.2 Nitrogen-balance and Blood Sampling

The experimental period consisted of a 7-d dietary adaptation period followed by a 4-d collection period. During the 4-d collection period, urine was collected quantitively daily over 24-h periods for each pig using metal trays and jugs placed underneath the metabolism crates. The jugs contained a sufficient amount of 6N HCl to minimize nitrogen losses by maintaining the pH below 3 (de Lange et al., 2001). Following each daily collection, total urine was weighed and a 10% subsample was obtained for each pig. Urine subsamples were pooled per pig over the collection period and stored at -20°C until further analysis. Fresh fecal grab samples were collected daily and stored at -20°C. At the end of each collection period, fecal samples were thawed, pooled for each pig, and homogenized. Subsamples were then stored at -20 °C until further analysis. Blood samples were collected from all pigs 2h after the morning meal on d2 of the collection period. Samples were obtained via jugular puncture into vacutainer tubes (BD, Vacutainers Mississauga, ON, Canada) containing heparin. Samples were then centrifuged at 2500 × *g* for 15 min, after which plasma was collected and stored at -20 °C for analysis of plasma urea nitrogen (PUN) and plasma amino acids.

4.3.3 Analytical Procedures

The dry matter (DM) content of the diets and fecal samples was analyzed in duplicate by oven drying at 135 ^{o}C for 2h (forced air ovens, Thermo Fisher Scientific Isotemp 750F). Fecal samples were freeze dried (Labconco Freeze Dry System, 18L; AOAC, 2007) before grinding in a centrifugal mill (Grinder Retsch ZM 200 2014) through a 1-mm sieve. Nitrogen content was determined in diet, fecal, and urine samples using an automatic analyzer (LECO FP 528; LECO FP 828; MI, USA; Method 990.03; AOAC, 2007). Titanium dioxide was determined as described by Myers et al. (2004). Diet samples were analyzed for AA composition at Central Testing Laboratories (Winnipeg, MB, Canada; Table 4.2). Plasma AA were analyzed by derivatization using the ACCQTag Ultra derivatization kit (Waters Corporation, Milford, MA, USA), followed by separation using UPLC with UV detection (Boogers et al., 2008). Plasma urea nitrogen was analyzed using a commercially available kit (Invitrogen Urea Nitrogen Colorimetric Detection Kit #EIABUN (BUN), Thermo Fisher Scientific, Waltham, MA, USA).

4.3.4 Calculations

Apparent total tract digestibility (ATTD) of N was determined using the indicator method according to the following equation:

N digestibility (ATTD) = $100 - [\frac{TiO2D \times NF}{TiO2F \times ND}] \times 100\%$

TiO_{2D} and TiO_{2F} are the titanium dioxide concentrations in the diet and feces, respectively. N_D and N_F are the nitrogen concentration in the diet and feces, respectively.

Nitrogen retention was determined using the following equation:

N retained (g/d) = N intake (g/d) – (fecal N output + urinary N output)

Protein deposition (g/d) was calculated as N retained × 6.25.

4.3.5 Statistical Analysis

Statistical analyses were conducted as a 2×5 factorial in a randomized complete block design using the MIXED model procedure of SAS (SAS Studio; SAS Institute, Inc. Cary, NC). The UNIVARIATE procedure of SAS was used to verify residual normality and identify outliers. Urine, fecal, and blood parameters were analyzed with dietary Lys (n = 5), ratio (n = 2), and their interactions as fixed effects and block (n = 8) as a random effect. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of dietary Lys inclusion on NR. Quadratic breakpoint modeling was conducted using PROC NLIN to estimate Lys requirement. PROC REG was used for regression analysis to determine the differences in efficiency of NR (slopes) between ratios. The Tukey-Kramer mean separation test was used to determine significant differences. The significance level was defined as $P \leq 0.05$.

4.4 Results

4.4.1 Nitrogen Balance

Nitrogen balance data are presented in Table 4.3. Initial body weight was greater in pigs fed the HR diets and increased with increasing dietary Lys content ($P < 0.05$). Nitrogen intake was greater with HR diets and generally increased with Lys content ($P < 0.05$). Fecal and urinary N output were lower in pigs fed the HR diets ($P < 0.01$), and urinary N output was decreased with increasing Lys content ($P < 0.01$). % NR of intake, NR, and PD increased in both groups with increasing Lys level ($P < 0.01$). There was no impact of dietary Lys content on fecal N output ($P > 0.05$). In general, NR, as % and g/d, and PD increased in LR-fed pigs compared to HR-fed pigs and increased with increasing dietary Lys content ($P < 0.05$). There was no effect of ratio on PUN concentration; however, PUN decreased with increasing Lys content ($P < 0.05$).

Breakpoint analysis determined a Lys requirement of 1.21% SID at 17.8 g/d NR (Fig 4.1A), 1.19% SID at 71.3% NR (Fig 4.2A), and 1.16% SID at 5.43 mg/dL PUN (Fig 4.3A) in pigs fed the HR diets. No breakpoint was achieved in pigs fed the LR diets (Fig 4.1B, 4.2B, 4.3B).

4.4.2 Plasma Amino Acids and Urea Nitrogen

Plasma amino acid concentrations are presented in Table 4.4. There was a significant effect of ratio on the plasma content of the EAA Leu, Lys, Met, Trp, and Val concentrations, with their concentrations greater in pigs fed the HR diets, except for Leu which was lower ($P < 0.05$). The concentrations of the NEAA Asp, Gly, and Ser were greater in pigs fed the LR diets compared to those fed the HR diets ($P < 0.01$). Increasing dietary Lys content resulted in an increase in plasma Lys concentration ($P < 0.01$) and a decrease in EAA concentrations of His, Ile, Phe, Thr, Tyr, and Val ($P < 0.05$). The NEAA Asn, Gln, Gly, and Pro concentrations were affected by dietary Lys content, with Gln and Gly concentrations increasing with increasing Lys ($P < 0.01$), and Asn and Pro concentrations decreasing with increasing Lys level ($P < 0.05$). There was an interactive effect of ratio and Lys on EAA Lys, and Thr ($P < 0.05$), with Lys concentration being highest and Thr being lowest in pigs fed the HR diet with 1.22% SID Lys. Plasma concentration of NEAA Cys was lowest in pigs fed HR diets with 0.82% SID Lys, and pigs fed the LR diets with 1.22% SID Lys ($P < 0.05$).

The interaction between ratio and Lys had an effect on the total plasma concentrations of EAA, with their concentration lowest in pigs fed the LR diet at a Lys level of 1.22% SID ($P = 0.01$). There were no effects of ratio, Lys, or their interaction on the plasma concentrations of total NEAA and total AA ($P > 0.05$).

4.4.3 Efficiency of Lys Intake for Nitrogen Retention

Efficiency data are presented in Figure 4.4. Efficiency of Lys utilization for NR (g/d) was greater in pigs fed LR vs. HR diets ($P < 0.05$). There was no effect of ratio on NR at maintenance requirement ($P > 0.05$).

4.5 Discussion

Due to the increased cost of protein and the excess protein provided in HP diets, nutritionists incorporate the feeding of LP diets with supplementation of CAA to pigs. Low protein diets improve gut health, immune status, and N utilization efficiency, and reduce N excretion into the environment (Nyachoti et al., 2006; van der Meer et al., 2016). However, growth performance may be negatively affected if dietary protein is reduced by more than 4% units from a typical protein content of 17.6%, even with an appropriate amount of EAA supplementation (Gloaguen et al., 2014). This indicates that NEAA or TN may be limiting in LP diets (Gloaguen et al., 2014; Mansilla et al., 2017a). In general, NEAA requirements have not been considered in diet formulations as it has been assumed that pigs are capable of synthesizing sufficient amounts of NEAA to meet their needs. However, a source of N is required for the endogenous synthesis of NEAA, and EAA may be used to provide N in N-deficient LP diets. This would reduce EAA availability and utilization efficiency for protein synthesis and may result in increased EAA requirements in LP diets. It is possible that the EAA-N:TN ratio may be used to reflect the sufficiency of dietary N supply in order to maximize N utilization efficiency and growth performance. In the present study, it was hypothesized that the Lys requirement would be increased in pigs fed diets limiting in N (i.e., high EAA-N:TN ratio).

An N-balance study was conducted to investigate the effects of feeding an optimal EAA-N:TN ratio of 0.48 or an N-limiting diet with an EAA-N:TN ratio of 0.55 on the Lys requirement for NR in 25 kg growing barrows. The results of the present study indicate an increase in NR, in g/d and as a % of N intake, with increasing Lys, regardless of EAA-N:TN ratio, with breakpoints of 1.21% and 1.19% SID Lys, respectively, achieved in HR diets. This is indicative of NEAA or N becoming limiting in HR diets. In contrast, a breakpoint was not achieved when pigs were fed an optimal EAA-N:TN ratio, indicating that NEAA or N were not limiting in these diets, and insufficient Lys was provided for the pigs to achieve maximum NR and to determine a requirement for Lys in LR diets. Similar results were observed when PUN was used to determine Lys requirement. Decreasing PUN levels with increasing SID Lys indicate improved N efficiency (Lopez et al., 1994; Kerr and Easter, 1995). Pigs fed the LR diets had greater NR, PD, and decreased PUN compared to pigs fed the HR diets, supported by the observed efficiency of Lys intake for NR. Similar NR results were observed in a study by Mansilla et al. (2017b) where growing pigs fed higher levels of ammonia-N (EAA-N:TN ratio of 0.50) had greater NR than pigs fed lower levels of ammonia-N (EAA-N:TN ratio of 0.59). Nitrogen retention values were slightly lower than the ones observed in the present study, which may be due to the lower dietary N intake (g/d) in the study by Mansilla et al. (2017b). The results of the present study provide further evidence for the appropriateness of an optimal ratio of 0.48 as observed by Heger et al. (1998).

It is possible that pigs may utilize the N from Lys and other EAA to produce NEAA, restricting the amount of EAA available for growth. Heger et al. (1998) observed that as the amount of EAA-N is increased, the utilization of EAA by the pig and other species for NR was decreased. They suggested that this is likely due to EAA-N being utilized to produce NEAA. Consequently, the provision of additional N in HR diets may improve NR. Millet et al. (2018a) conducted a study in weanling piglets investigating the effects of feeding diets with different SID Lys:CP ratios. They observed that the effect of decreasing dietary CP is dependent on SID Lys, and once a ratio of SID Lys:CP of 0.064 is exceeded, overall protein (N) rather than an individual AA is limiting (Millet et al., 2018a). At this point, it is suggested that EAA may be used to produce NEAA, which is in agreement with our current study where N or NEAA are limiting in HR diets. In the current study, no breakpoint was achieved for NR in pigs fed LR diets, indicating sufficient N but insufficient Lys provision to maximize growth. Similar results were observed in the study by Heger et al. (1998), where pigs had low utilization of TN when fed LR diets, which may be due to either a lack of dietary EAA, when the TN fraction was kept constant, or due to the conversion of EAA-N into NEAA, when the EAA-N fraction was kept constant, as in the present study. Further evidence for the utilization of EAA, and specifically Lys, for NEAA synthesis is provided by the efficiency of NR obtained in the current study, which was improved in pigs fed LR diets, which were formulated with the suggested optimal ratio of 0.48.

The concentrations of the EAA Lys, Met, Trp, and Val all increased in pigs fed HR diets. These increases may be due to the pig being deficient in N and unable to utilize these EAA for protein deposition, resulting in increased plasma concentrations. There was a decrease in the concentration of Leu in pigs fed the HR diets, which may be related to a decrease in protein synthesis due to the limited amount of N available for NEAA synthesis (Duan et al., 2015). The lower plasma concentrations of the EAA Lys, Met, Trp, and Val in pigs fed the LR diets may be explained by an increase in their utilization due to the provision of sufficient N. An increase in Lys resulted in decreased plasma concentrations of the EAA His, Ile, Phe, Thr, Tyr, and Val which may indicate improved NR, which may be due to an increase in protein synthesis due to the provision of Lys (Everson et al., 1989; Roy et al., 2000). Similar results were observed in a study by Roy et al. (2000), where the concentrations of His, Ile, Thr, and Val decreased with increasing Lys level. Total EAA were highest in the LR diets at the lowest inclusion of dietary Lys, which may be indicative of EAA not being utilized for PD due to the lack of Lys available. The most limiting AA for growth in cereal-soybean meal diets are Lys, Met, Thr, and Trp with Val, Ile, and His thought to be the next limiting, which supports the results of the present study (Figueroa et al., 2003; Kerr et al., 2003).

The increase in the concentrations of the NEAA Asp, Gly, and Ser in pigs fed the LR is consistent with the hypothesis of the present study as LR diets provide more TN, largely in the form of NEAA. Since more N is available to the pig with the provision of LR diets, it is possible that the increase in Gly and Ser is due to the utilization of ammonia via the glycine synthetase reaction to produce Gly, which may then be converted to Ser via the serine hydroxymethyl transferase reaction (Young and Ajami, 2001). The increase in Asp may be indicative of the increase of TN with the LR diets, as AA are catabolized to Glu which is transaminated to produce Asp (Berg et al., 2002). The lower concentrations of the NEAA Asp, Gly, and Ser in pigs fed the HR diets may be indicative of their low dietary supply or insufficient endogenous synthesis due to a N deficiency. The plasma concentration of Arg was decreased in pigs fed the LR diets, which could indicate its utilization in the urea cycle to produce ornithine, as more N is present in these diets and may be excreted as urea if not utilized (Meijer et al., 1990). There was also an observed increase in Gln and Gly and a decrease in Asn and Pro with increasing Lys. Lysine acts as a ketogenic AA and is converted to acetyl CoA, which can be incorporated into the Krebs Cycle to produce energy (Matthews, 2020). The synthesis of Gln requires energy, so it is possible that an increase in energy production with increased dietary Lys enables Gln production. An increase in Lys may also result in increased AA catabolism if in excess, consequently Gln is produced as an N carrier. In addition, since an increase in dietary Lys resulted in increased NR in LR diets, the increase in certain NEAA may correlate with an increase in protein synthesis.

The decreased plasma Thr concentration in LR diets at the highest Lys inclusion when compared to HR diets may be indicative of its utilization for protein synthesis and, therefore, growth. As Thr is one of the most limiting AA for growth, increasing Lys content in LR diets may improve its utilization. There was a general increase in plasma Cys concentration in pigs fed the HR diets with increasing Lys, and a general decrease in pigs fed the LR diets with increasing Lys. This may be indicative of its low utilization in HR diets and increased utilization in LR diets.

In conclusion, Lys requirement was demonstrated to be higher than current NRC (2012) requirements. In addition, the results from the present study indicate that NEAA or N becomes limiting in HR diets but not in LR diets, and more EAA are required in LR diets to meet requirements for PD and NR. The ratio of EAA-N:TN should be considered in LP diet formulation.

Table 4.1. Ingredient composition of experimental diets[1] (as-fed basis)

Ingredient, %	**High Ratio**		**Low Ratio**	
Dietary Lys, % SID	0.82	1.22	0.82	1.22
Soybean Meal	5.0	5.0	10.0	10.0
Corn	79.9	79.4	75.7	75.2
Soy protein concentrate	8.0	8.0	8.0	8.0
Soybean oil	2.5	2.5	2.5	2.5
L-Lysine	0.297	0.807	0.135	0.645
L-Arginine	0.150	0.150	-	-
DL-Methionine	0.153	0.153	0.111	0.111
L-Threonine	0.242	0.242	0.172	0.172
L-Tryptophan	0.072	0.072	0.040	0.040
L-Isoleucine	0.091	0.091	-	-
L-Valine	0.082	0.082	0.002	0.002
L-Histidine	0.053	0.053	-	-
Monocalcium phosphate	1.38	1.38	1.32	1.32
Limestone	1.32	1.32	1.32	1.32
Salt	0.40	0.40	0.40	0.40
Titanium dioxide	0.10	0.10	0.10	0.10
Vitamin/mineral premix[2]	0.20	0.20	0.20	0.20
***Calculated nutrient content*[3]**				
EAA-N:TN[4]	0.55	0.55	0.48	0.48
DM, %	87.7	89.1	88.3	88.2
CP, %	15.3	15.7	16.6	17.0
ME, kcal/kg	3461	3466	3449	3454
NE, kcal/kg	2663	2667	2630	2634
Ca (%)	0.77	0.77	0.77	0.77
P (%)	0.38	0.38	0.38	0.38
Amino Acids, % SID				
Lys	0.82	1.22	0.82	1.22
Arg	0.95	0.95	0.95	0.95
His	0.40	0.40	0.40	0.40
Ile	0.59	0.59	0.59	0.59
Leu	1.20	1.20	1.32	1.32
Met+Cys	0.58	0.58	0.58	0.58
Phe+Tyr	1.02	1.02	1.17	1.17
Thr	0.67	0.67	0.67	0.67
Trp	0.19	0.19	0.19	0.19
Val	0.67	0.67	0.67	0.67

CP, crude protein; DM, dry matter; EAA-N, essential amino acid nitrogen; ME, metabolizable energy; NE, net energy; SID, standardized ileal digestible; TN, total nitrogen.

[1] The lowest and highest Lys diets were blended in appropriate proportions to achieve diets containing the other graded levels of Lys (not shown).

[2] Supplied per kilogram of complete feed: vitamin A, 4 000 IU; vitamin D, 0.019 mg; vitamin E, 15 IU; vitamin B12, 0.01 mg; menadione, 1.0 mg; thiamine, 0.50 mg; riboflavin, 2.0 mg; pyridoxine, 1.0 mg; niacin, 10 mg; pantothenate, 6 mg; folic acid, 0.25 mg; biotin, 0.05 mg; Cu, 7.5 mg; Fe, 50 mg; Mg, 20 mg; I, 0.50 mg; Zn, 50 mg, and Se, 0.15 mg.

[3] Nutrient content of diets based on estimated nutrient contents of feed ingredients according to NRC (2012).

[4] EAA-N:TN ratios of 0.55 (high ratio diets) and 0.48 (low ratio diets) reflect the amount of nitrogen in the diets coming from essential amino acids (EAA-N) and from the other components (TN), with the higher ratio having a larger contribution of nitrogen from EAA.

Table 4.2. Analyzed nutrient content of experimental diets (as-fed basis)[1]

	High Ratio					**Low Ratio**				
Lys, % SID	**0.82**	**0.92**	**1.02**	**1.12**	**1.22**	**0.82**	**0.92**	**1.02**	**1.12**	**1.22**
DM, %	90.2 (87.0)	90.1 (87.0)	90.6 (87.0)	90.5 (87.0)	90.3 (87.0)	90.3 (87.0)	90.1 (87.0)	90.5 (87.0)	90.6 (87.0)	90.3 (87.1)
CP, %	16.0 (15.3)	15.7 (15.4)	16.4 (15.5)	16.4 (15.6)	16.6 (15.8)	17.1 (16.6)	17.3 (16.7)	17.2 (16.8)	17.1 (16.9)	17.2 (17.))
Total Amino Acid, %										
Lys	0.78 (0.91)	0.8 (1.02)	0.96 (1.12)	1.17 (1.22)	1.19 (1.31)	0.90 (0.92)	0.96 (1.03)	1.08 (1.13)	1.18 (1.23)	1.22 (1.32)
Met	0.36 (0.40)	0.37 (0.41)	0.31 (0.41)	0.29 (0.41)	0.33 (0.40)	0.31 (0.38)	0.35 (0.39)	0.25 (0.39)	0.34 (0.39)	0.29 (0.38)
Met+Cys	0.61 (0.66)	0.62 (0.67)	0.50 (0.67)	0.51 (0.67)	0.54 (0.66)	0.55 (0.67)	0.63 (0.68)	0.43 (0.68)	0.61 (0.68)	0.54 (0.66)
Thr	0.54 (0.76)	0.49 (0.71)	0.54 (0.70)	0.64 (0.70)	0.59 (0.76)	0.62 (0.77)	0.63 (0.72)	0.61 (0.72)	0.63 (0.72)	0.59 (0.77)
Arg	0.84 (0.85)	0.81 (0.85)	0.80 (0.85)	0.92 (0.85)	0.92 (0.85)	0.90 (1.01)	0.90 (1.00)	0.89 (1.00)	0.91 (1.00)	0.87 (1.00)
Ile	0.62 (0.65)	0.61 (0.65)	0.62 (0.65)	0.71 (0.65)	0.70 (0.65)	0.72 (0.67)	0.70 (0.66)	0.72 (0.66)	0.72 (0.66)	0.69 (0.66)
Leu	1.31 (1.36)	1.26 (1.36)	1.28 (1.36)	1.46 (1.36)	1.45 (1.36)	1.58 (1.50)	1.55 (1.50)	1.58 (1.50)	1.58 (1.50)	1.54 (1.50)
Val	0.72 (0.75)	0.71 (0.77)	0.72 (0.76)	0.83 (0.76)	0.81 (0.75)	0.82 (0.76)	0.80 (0.77)	0.82 (0.77)	0.82 (0.72)	0.80 (0.76)
His	0.35 (0.44)	0.35 (0.44)	0.37 (0.44)	0.43 (0.44)	0.36 (0.44)	0.41 (0.45)	0.42 (0.45)	0.37 (0.45)	0.43 (0.44)	0.41 (0.44)
Phe	0.66 (0.70)	0.65 (0.70)	0.65 (0.70)	0.76 (0.70)	0.76 (0.70)	0.82 (0.81)	0.83 (0.81)	0.82 (0.80)	0.84 (0.80)	0.81 (0.80)

Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Met+Cys, methionine + cysteine; Phe, phenylalanine; SID, standardized ileal digestible; Thr, threonine; Val, valine.

[1] Analyzed total amino acid content with calculated values in parentheses.

Table 4.3. Nitrogen balance in pigs fed a low EAA-N:TN ratio (LR) or high EAA-N:TN ratio (HR) diets with graded levels of lysine[1]

		SID Lysine, %						***P*-value**		
Item	**Ratio**	**0.82**	**0.92**	**1.02**	**1.12**	**1.22**	**SEM[3]**	**Ratio**	**Lys**	**Ratio×Lys**
Initial body weight, kg	0.55	20.6	21.4	21.7	21.5	21.3	0.23	0.02	<0.01	0.44
	0.48	21.0	21.6	22.1	21.4	22.1				
N intake, g/d	0.55	24.4	24.3	24.7	25.3	25.0	0.13	<0.01	<0.01	<0.01
	0.48	26.1	25.8	26.4	26.1	26.9				
Fecal N output, g/d	0.55	4.2	3.8	4.3	4.3	4.5	0.19	<0.01	0.40	0.30
	0.48	4.7	4.9	4.8	4.8	5.0				
Urinary N output, g/d	0.55	4.9	4.1	3.6	3.0	2.5	0.20	<0.01	<0.01	0.43
	0.48	6.1	5.1	4.2	3.5	3.2				
ATTD of N, %	0.55	83.4	84.3	82.5	83.3	81.7	0.69	<0.01	0.81	0.21
	0.48	81.4	81.2	81.8	81.9	82.0				
N retained, % of N intake	0.55	54.3	57.8	62.1	64.1	62.9	1.12	<0.01	<0.01	0.10
	0.48	48.8	53.1	60.2	58.8	62.9				
N retained, g/d	0.55	14.9	16.0	17.1	17.9	17.8	0.26	0.02	<0.01	<0.01
	0.48	15.2	15.8	17.9	17.4	19.3				
PD[2], g/d	0.55	93.2	99.9	106.9	111.9	111.3	1.60	0.02	<0.01	<0.01
	0.48	94.8	98.9	111.9	109.0	120.8				
PUN, mg/dL	0.55	10.8	9.2	6.8	2.8	7.6	1.54	0.51	<0.01	0.48
	0.48	9.3	11.2	6.0	3.3	4.0				

ATTD; apparent total tract digestibility; EAA-N:TN, essential amino acid nitrogen to total nitrogen ratio; HR, high ratio; LR, low ratio; Lys, lysine; N, nitrogen; PD, protein deposition; PUN, plasma urea nitrogen; SEM, standard error of the mean; SID, standardized ileal digestible.

[1] Data presented are least-square means (*n*=8 pigs/treatment)

[2] PD calculated as N retained (g/d) × 6.25

[3] Average SEM per parameter.

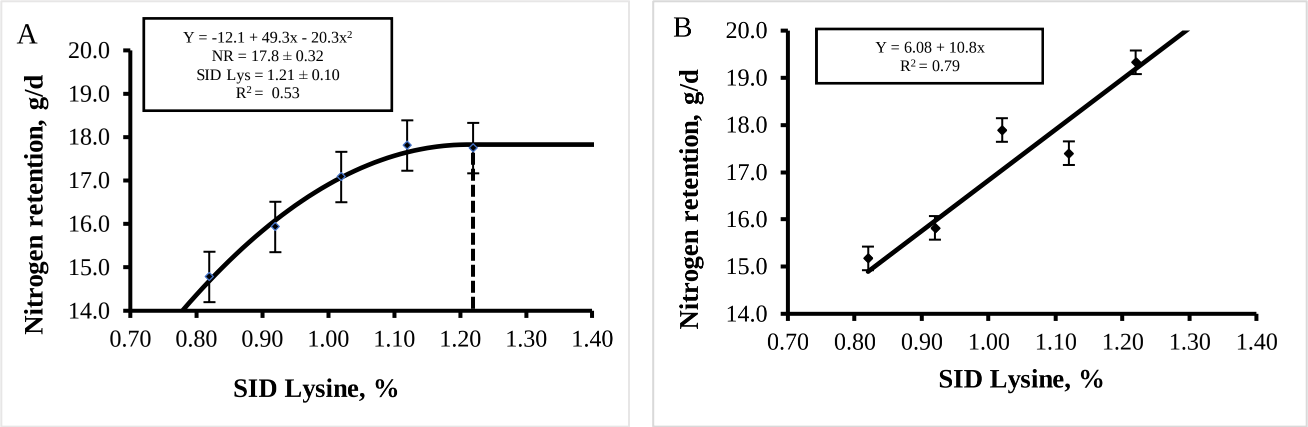

Figure 4.1. The quadratic breakpoint and linear model analyses estimates for nitrogen retention (NR; g/d) in pigs fed high essential amino acid-nitrogen:total nitrogen ratio (HR; 0.55; A) or low ratio (LR; 0.48; B) diets. The analyses indicate a breakpoint was achieved at 1.21% with maximum NR at 17.8 g/d for pigs fed the HR diet. No breakpoint was achieved in pigs fed the LR diet.

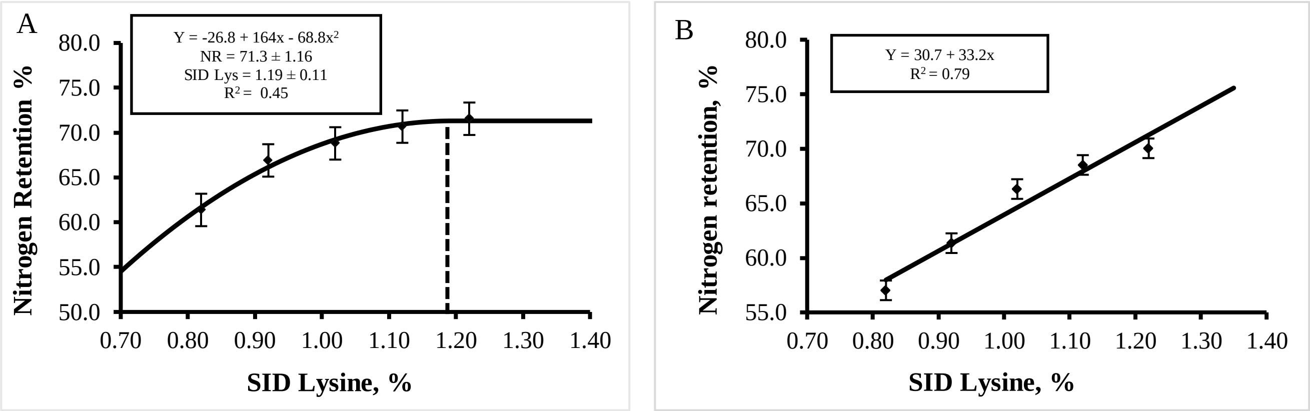

Figure 4.2. The quadratic breakpoint and linear model analyses estimates for nitrogen retention (NR; %) in pigs fed a high essential amino acid-nitrogen:total nitrogen ratio (HR; A) or low ratio (LR; B) diets. A breakpoint was achieved at 1.19% in pigs fed the HR diet, with maximum NR at 71.3%. No breakpoint was achieved in pigs fed the LR diet.

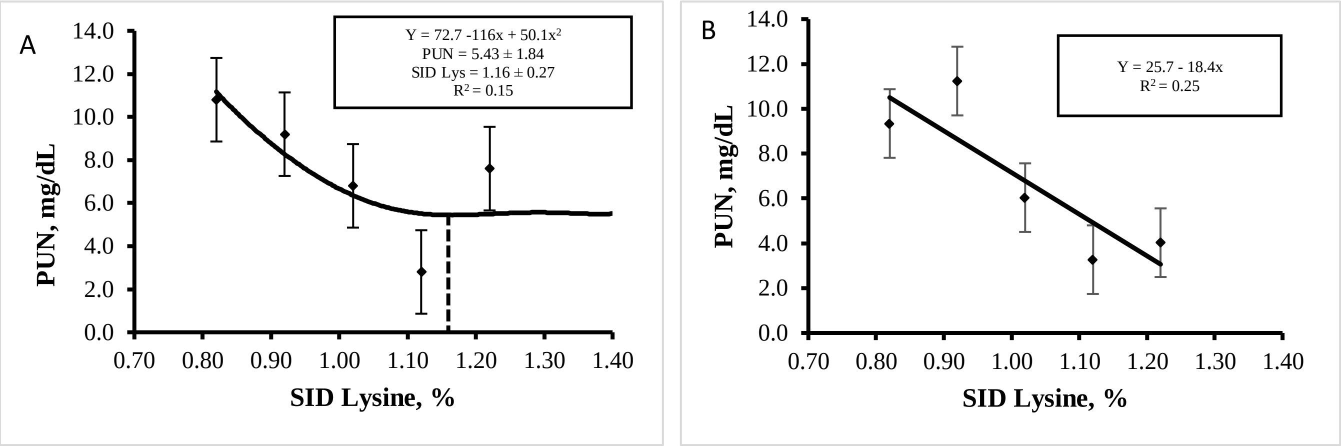

Figure 4.3. The quadratic breakpoint and linear model estimates for plasma urea nitrogen (PUN; mg/dL) in pigs fed a high essential amino acid-nitrogen:total nitrogen ratio (HR; A) or a low ratio (LR; B) diet. A breakpoint was achieved at 1.16% with a value of 5.43 mg/dL in pigs fed the HR diet. No breakpoint was achieved in pigs fed the LR diet.

Table 4.4. Plasma amino acid concentrations (μM) in pigs fed low EAA-N:TN ratio (LR) or high EAA-N:TN ratio (HR) diets with graded levels of Lys[1,2,3]

		Lysine, % SID						***P*-value**		
Item	**Ratio**	**0.82**	**0.92**	**1.02**	**1.12**	**1.22**	**SEM[4]**	**Ratio**	**Lys**	**Ratio × Lys**
Essential Amino Acids										
His	0.55	106	79	84	71	61	4.3	0.06	<0.01	0.39
	0.48	104	84	72	61	49				
Ile	0.55	144	125	124	94	121	8.8	0.06	<0.01	0.17
	0.48	128	115	111	108	83				
Leu	0.55	217	196	199	200	203	15.1	0.05	0.56	0.93
	0.48	237	219	215	231	208				
Lys	0.55	205	199	256	344	438	20.6	<0.01	<0.01	0.03
	0.48	176	182	226	278	291				
Met	0.55	88	79	90	94	82	6.9	<0.01	0.31	0.46
	0.48	71	76	86	70	65				
Phe	0.55	105	85	101	86	86	6.3	0.30	<0.01	0.16
	0.48	117	102	89	95	81				
Thr	0.55	626	499	444	406	318	46.2	0.67	<0.01	0.01
	0.48	837	571	385	345	222				
Trp	0.55	82	64	78	75	56	5.6	0.03	0.06	0.32
	0.48	66	66	56	67	56				
Tyr	0.55	135	96	105	97	97	8.7	0.24	0.03	0.59
	0.48	125	115	116	114	99				
Val	0.55	283	267	278	248	250	16.0	<0.01	<0.01	0.09
	0.48	285	252	244	203	148				
TEAA	0.55	1995	1694	1764	1719	1717	105.3	0.02	<0.01	0.01
	0.48	2150	1786	1603	1577	1306				
Non-Essential Amino Acids										
Ala	0.55	701	661	652	720	619	48.0	0.14	0.42	0.99
	0.48	749	684	643	743	684				
Arg	0.55	259	222	249	248	209	16.0	<0.01	0.12	0.75
	0.48	233	206	209	185	184				
Asn	0.55	109	90	89	96	89	7.2	0.07	0.04	0.96
	0.48	114	101	100	108	93				
Asp	0.55	18	21	21	22	17	2.1	<0.01	0.61	0.27
	0.48	28	21	21	26	23				
Cys	0.55	7	21	11	15	11	2.4	0.68	0.06	0.02
	0.48	17	14	15	10	6				
Gln	0.55	702	729	717	900	778	51.3	0.90	<0.01	0.57
	0.48	711	681	733	846	874				
Glu	0.55	245	292	289	320	281	20.0	0.88	0.58	0.17
	0.48	298	265	269	282	324				
Gly	0.55	1142	1098	1150	1317	1305	62.5	<0.01	<0.01	0.73
	0.48	1228	1207	1198	1433	1525				
Pro	0.55	439	392	391	412	411	22.9	0.19	0.03	0.17
	0.48	498	467	406	427	360				

Ser	0.55	170	154	151	158	154	12.2	<0.01	0.27	0.79
	0.48	195	177	160	186	194				
TNEAA	0.55	3795	3685	3725	4211	3877	151.8	0.08	0.15	0.50
	0.48	4075	3827	3757	4250	4272				
TAA	0.55	5791	5379	5489	5931	5595	243.0	0.89	0.45	0.51
	0.48	6225	5614	5360	5827	5579				

EAA-N:TN; essential amino acid-nitrogen to total nitrogen ratio; TAA, total amino acids; TEAA, total essential amino acids; TNEAA, total non-essential amino acids.

[1] Data presented as least-square means with

[2] Samples were obtained 2 h after the morning meal (0700 h) on d 2 of the collection period.

[3] EAA-N:TN ratios of 0.55 (high ratio diets) and 0.48 (low ratio diets) reflect the amount of nitrogen in the diets coming from essential amino acids (EAA-N) and from the other components (TN), with the higher ratio having a larger contribution of nitrogen from EAA.

[4] Pooled SEM

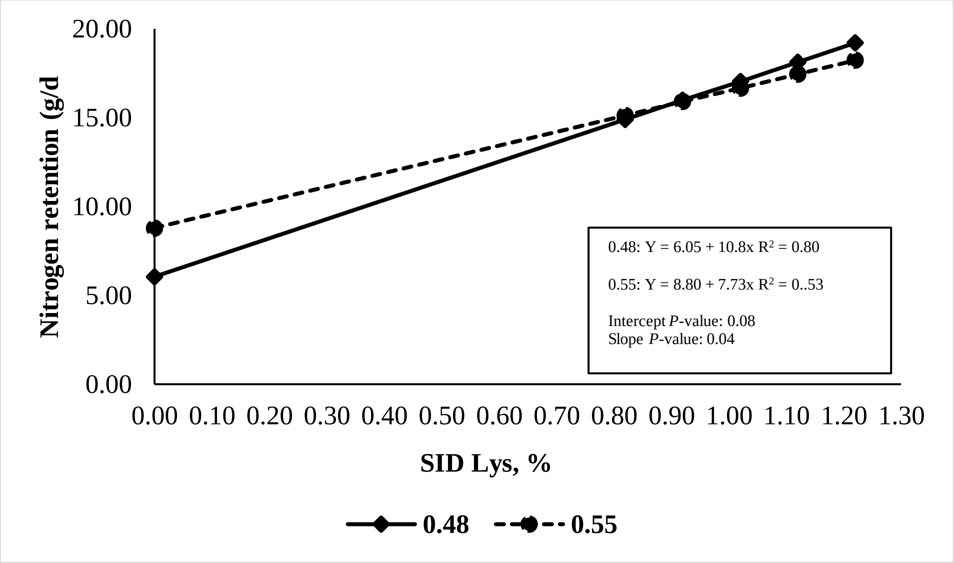

Figure 4.4. Efficiency of Lys intake for NR (g/d) in pigs fed a low essential amino acid-nitrogen:total nitrogen ratio (LR; 0.48) vs. a high ratio (HR; 0.55) diet.

5.0 THE EFFECT OF FEEDING A DIET WITH SUPPLEMENTAL LYSINE AND A LOW OR HIGH ESSENTIAL AMINO ACID-NITROGEN TO TOTAL NITROGEN RATIO ON GROWTH PERFORMANCE IN 20-50 KG PIGS

5.1 Abstract

Nitrogen (**N**) or non-essential amino acids (**NEAA**) may limit growth performance when pigs are fed low protein (**LP**) diets due to the decrease in available dietary protein. The essential amino acid-nitrogen to total nitrogen (**EAA-N:TN**) ratio has been suggested to improve N utilization as it considers the amount of dietary N the animal requires for the endogenous synthesis of NEAA. Results from our previous study (Chapter 4) indicated that N or NEAA becomes limiting in high ratio (**HR**) diets, but not in low ratio (**LR**) diets, and that more lysine (**Lys**) than currently recommended is required in LR diets to maximize nitrogen retention (**NR**). A follow-up growth performance study was conducted to determine whether LP diets supplemented with Lys and deficient in total N (**TN**; or NEAA-N) affected growth performance and nutrient excretion in growing pigs. A total of 240 gilts and barrows (20.6 initial BW; SD = 2.03 kg) were randomly assigned to pens (*n* = 12/treatment) that had been randomly assigned to 1 of 4 dietary treatments (*n* = 60 pigs/treatment) in 3 blocks in a 2 × 2 factorial design. Diets consisted of a low ratio (**LR**; 17.4% crude protein; EAA-N:TN of 0.48) or a high ratio (**HR**; 16.3% crude protein; EAA-N:TN of 0.55) with graded levels of Lys (1.03% and 1.22% standardized ileal digestible [**SID**]). Pigs were fed ad libitum, with feed intake and individual pig body weight measured weekly to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) over an experimental period of 28 d. Data were analyzed using PROC MIXED with fixed effects of ratio, lysine, and their interaction and block as a random effect. Increasing Lys level resulted in increased overall ADG and ADFI ($P < 0.01$), but there was no effect of ratio. Pigs fed the HR diets had improved ADG in week 4 ($P < 0.01$), whereas pigs fed the LR diets had improved ADG and G:F in week 1 ($P < 0.01$). There was an interactive effect of ratio and Lys on N output ($P < 0.01$), where N output decreased only in pigs fed the HR diets with 1.22% Lys. These results indicate that insufficient dietary N may be more of a concern during periods of general nutrient insufficiency. In addition, the current recommended Lys levels may not be sufficient to maximize growth in 20-50 kg pigs being fed diets deficient in dietary N.

5.2 Introduction

Low protein (**LP**) diets supplemented with essential amino acids (**EAA**) have been successful in improving the animal's N utilization efficiency (Gloaguen et al., 2014). However, if dietary crude protein levels are reduced by more than 4% units from 17.6% CP, performance may be negatively affected (Gloaguen et al., 2014). At this point, it is suggested that nitrogen (**N**) or non-essential amino acids (**NEAA**) are limiting growth since less dietary N is available to the animal for the endogenous synthesis of NEAA (Mansilla et al., 2017a). This theory is supported as dietary supplementation of NEAA or N have improved growth performance in growing pigs fed LP diets (Heger et al., 1998; Powell et al., 2011).

The EAA-N to total N (**EAA-N:TN**) ratio has been introduced as it represents the N that the animal is receiving from dietary EAA and total dietary N (Heger et al., 1998). The EAA-N:TN ratio be utilized as an indicator of the appropriate amount of EAA and N being provided in the diet (Heger et al., 1998; Wang & Fuller, 1989). In a diet deficient in N, any excess EAA supplied may be used for the endogenous synthesis of NEAA and would be considered as part of the TN fraction of the ratio (Heger, 2003). Results from our previous study indicated that N or NEAA become limiting in high ratio (HR) diets, whereas sufficient N was supplied in LR diets. We also demonstrated that Lys that the lysine requirement in both LR and HR diets was greater than estimated by NRC (2012). Consequently, the objective of this study was to determine the growth performance of 20-50 kg pigs fed diets sufficient in TN or deficient in TN (or NEAA-N) with Lys included at or above current requirements (NRC, 2012). It was hypothesized that growth performance would be improved with increasing Lys level and in pigs fed LR diets.

5.3 Materials and Methods

The experimental protocol was reviewed and approved by the University of Saskatchewan's Animal Research Ethics Board (AUP #20130054) and followed the Canadian Council on Animal Care guidelines (CCAC, 2009).

5.3.1 Animals, Diet, and Experimental Design

A total of 240 grower pigs of 20.6 ± 2.03 kg initial body weight were used in a growth performance study at the Prairie Swine Centre, Inc (Saskatoon, SK, Canada). Pigs were randomly grouped into pens of 5 pigs/pen (either 2 barrows and 3 gilts, or 3 barrows and 2 gilts) over 3 blocks. Pens were randomly assigned (*n*=12/treatment) to 1 of 4 dietary treatments (*n*=60/treatment). The treatments were arranged as a 2 × 2 factorial design with factors of ratio (low EAA-N:TN of 0.48 or high EAA-N:TN of 0.55) and dietary lysine level [at 1.03% SID Lys or 1.22% SID Lys representing NRC (2012) requirement or the previously determined requirement (Chapter 4), respectively]. The diets were corn and soybean meal-based (Table 5.1) and celite was included as an indigestible marker to determine apparent total tract digestibility (ATTD). Diets were formulated to meet or exceed all other nutrient requirements (NRC, 2012) for 20-50 kg pigs. Pigs were fed ad libitum and had free access to water for the entirety of the study.

5.3.2 Experimental Procedure and Nutrient Analyses

Pigs were weighed at the start of the experiment (d 0), and on d 7, 14, 21, and 28 to calculate average daily gain (ADG). Feed added and feed refusals were monitored weekly and used to calculate average daily feed intake (ADFI) and gain:feed (G:F). Fresh fecal samples were collected from 2 - 3 pigs per pen on d 14 of the experiment to determine N digestibility. Samples were pooled during collection, homogenized, and frozen at -20 °C until further analysis. Fecal samples were freeze dried (Labconco Freeze Dry System, 18L; AOAC, 2007) for 5 d before grinding in a centrifugal mill (Grinder Retsch ZM 200 2014) through a 1-mm sieve. Fecal and feed samples were analyzed for N content using an automatic analyzer (LECO FP 528; MI, USA; Method 990.03; AOAC, 2007). Diet samples were analyzed for AA composition at Central Testing Laboratories (Winnipeg, MB, Canada; Table 5.2). Diet and fecal samples were analyzed in duplicate for acid insoluble ash (AIA) as described by Van Keulen and Young (1977) to determine N digestibility, calculated as follows:

N digestibility (ATTD) = $100 - [\frac{AIAD \times NF}{AIAF \times ND}] \times 100\%$

Where AIA_D and AIA_F are the AIA concentrations of the diet and feces, respectively, and N_D and N_F are the N concentrations in the diet and feces, respectively. All concentrations were on a DM basis.

5.3.3 Statistical Analyses

Data were analyzed using the MIXED model procedure of SAS (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to verify data normality and to identify outliers. The MIXED model procedure consisted of ratio ($n = 2$), dietary Lys level ($n = 2$), and their interaction as fixed effects, and block ($n = 3$) as a random effect. The Tukey-Kramer mean separation test was used to determine significant differences. The significance level was defined as $P \leq 0.05$.

5.4 Results

5.4.1 Growth Performance and Apparent Total Tract Digestibility (%)

Three pigs were removed from the study due to injury or illness, and calculations were adjusted accordingly. Growth performance data are presented in Table 5.3. Increasing dietary Lys resulted in greater overall ADG ($P < 0.01$). There was an effect of ratio on ADG during week 1 and week 4 ($P < 0.05$). Pigs fed the LR diets had greater ADG during week 1, whereas pigs fed the HR diets had greater ADG during week 4. Overall ADFI was greater with increasing dietary Lys content ($P < 0.01$), but there was no effect of ratio on overall ADFI ($P = 0.42$). In addition, ADFI was greater starting in week 2 with increasing Lys content ($P < 0.05$). Inclusion of 1.03% SID Lys level resulted in reduced G:F ratio during week 1 ($P < 0.01$), but there were no other observed effects of Lys on G:F. Pigs fed the HR diets had reduced G:F ratio compared to pigs fed the LR diets during week 1 ($P < 0.01$). There were no differences between initial BW among dietary treatments; however, pigs fed the LR diets had a higher BW than pigs fed the HR diets on d 7 ($P < 0.05$). Increasing dietary Lys content resulted in a greater BW on d 7, 14, 21, and 28 ($P < 0.05$). There were no interactive effects of ratio and Lys on ADG, ADFI, G:F, or BW ($P > 0.05$).

The ATTD (%) of N was not affected by ratio or lysine, but there was a significant interactive effect of ratio and Lys on ATTD ($P < 0.01$), with ATTD of N being greatest in pigs fed the HR diet with Lys included at 1.22% SID (Table 5.4). Similarly, there was an interactive effect of ratio and Lys on fecal N output ($P < 0.01$), with a decrease in N output in pigs fed the HR diets with 1.22% SID Lys inclusion (Table 5.4).

5.5 Discussion

The aim of the present study was to investigate the growth performance of 20-50 kg pigs fed diets either sufficient or deficient in N, as indicated by the EAA-N:TN ratio and with Lys included to meet NRC (2012) requirement or to meet the requirement as determined previously (Chapter 4). It was hypothesized that growth performance would be improved in pigs receiving a diet containing sufficient N (EAA-N:TN ratio of 0.48) or in pigs receiving diets limiting in N (EAA-N:TN ratio of 0.55), but with supplemental Lys. The present study demonstrated that increasing dietary Lys resulted in increased ADG, ADFI, and a higher BW, regardless of ratio. In addition, pigs consuming the LR diets had greater ADG and ADFI and greater feed efficiency during week 1 of the experiment, whereas these were lower in pigs fed the HR diets during week 1. In contrast, pigs fed the HR diets had greater ADG during week 4, whereas those fed the LR diets had a lower ADG compared to pigs fed the HR diets during week 4. Average daily gain and G:F were not affected by ratio during other weeks. Traditional phase feeding programs involve periods of varying nutrient sufficiency, with nutrient supply generally insufficient at the start of the phase, but in excess at the end of the phase (Millet et al., 2018b). It is possible that insufficient N may have a greater impact on growth during periods of nutrient insufficiency, such as immediately following a dietary change during phase-feeding. As diets in the current study were formulated for 20-50 kg pigs, this may indicate that HR diets contained insufficient N during the early growth stage; however, this was not the case later in the study. Pigs fed the LR diets had greater ADG during week 1, but less ADG during week 4 when compared to pigs fed the HR diets, indicating that Lys may have been limiting and N in excess during the later growth phases. Pomar et al. (2021) stated that young pigs have improved efficiency when compared to older pigs, which may explain the greater ADG during week 1 in pigs fed LR diets in the current study, as they may have been able to utilize the EAA-N more efficiently. In contrast, the increase in ADG during week 4 in pigs fed the HR diets may be due to the sufficient amount of Lys available that was efficiently utilized until N or NEAA-N became limiting.

Greater ADG with increasing Lys throughout the experimental period is indicative of more Lys being used for protein deposition, regardless of ratio. Although greater ADG with increasing Lys is expected, these results confirm results obtained in our previous study (Chapter 4), indicating that the Lys requirement is higher than current NRC (2012) recommendations. Although pigs match their feed intake to their energy requirements, feed intake may also be regulated by gut capacity (Whittemore et al., 2003). The ADFI/BW ratios of the present study were similar across diets within week, indicating that the increase in ADFI observed with increasing SID Lys in the present study may be due to the effect of SID Lys on BW, with the pigs consuming more Lys having a heavier BW throughout the study. Similar results were obtained in a study by Remus et al. (2020), where ADFI increased with increasing SID Lys in 25-50 kg grower pigs. In addition, Rodriguez-Sanchez et al. (2011) conducted a study with finishing pigs and observed a decrease in ADFI with decreasing Lys, which may have been due to BW.

Furthermore, pigs fed the HR diets with increased Lys had decreased N output, which is likely due to the lower protein content of the HR diets. Heger et al. (1998) stated that increasing the EAA-N:TN ratio to the point that N or NEAA becomes limiting resulted in decreased N excretion, as seen in pigs fed the HR diets with increasing Lys in the current study. In addition, a decrease in N output was also observed in pigs fed LR diets with 1.03% SID Lys when compared to pigs fed LR diets with 1.22% SID Lys. Therefore, at an optimal ratio of 0.48, less Lys may be required to improve NR. Incorporating the concept of the EAA-N:TN into diet formulations would be beneficial to maximize NR by providing the appropriate amount of EAA and TN depending on whether the EAA-N:TN ratio is lower or higher. Diet formulations with accurate estimates of EAA and TN would be beneficial to producers as diet costs may decrease, and N excretion into the environment reduced.

In conclusion, this study presents results suggesting that current Lys recommendations may not be sufficient to maximize growth in 20-50 kg pigs and dietary Lys content may need to be increased in current LP diet formulations to allow the pig to maximize its growth potential. In addition, the appropriate provision of dietary N, especially during phases of nutrient (AA) insufficiency, should be considered for improved growth performance. The EAA-N:TN ratio may be beneficial in helping to maximize nutrient utilization and growth performance in LP diets.

Table 5.1. Ingredient and calculated nutrient composition of experimental diets (as-fed basis)

	High Ratio		**Low Ratio**	
Dietary Lys, % SID	**1.03**	**1.22**	**1.03**	**1.22**
Ingredients, %				
Soybean Meal	5.50	5.50	10.0	10.0
Corn	81.1	80.8	76.6	76.3
Soy Protein Concentrate	8.0	8.0	8.0	8.0
Soybean Oil	0.0	0.0	0.6	0.6
L-Lysine	0.55	0.80	0.41	0.66
L-Arginine	0.13	0.13	0.0	0.0
DL-Methionine	0.18	0.18	0.16	0.16
L-Threonine	0.29	0.29	0.23	0.23
L-Tryptophan	0.15	0.15	0.13	0.13
L-Isoleucine	0.13	0.13	0.06	0.06
L-Valine	0.19	0.19	0.11	0.11
L-Histidine	0.11	0.11	0.07	0.07
Monocalcium Phosphate	1.38	1.38	1.32	1.32
Limestone	1.32	1.32	1.32	1.32
Salt	0.4	0.4	0.4	0.4
Celite	0.4	0.4	0.4	0.4
Vitamin/mineral Premix[1]	0.2	0.2	0.2	0.2
Calculated nutrient content[2]				
EAA-N:TN[3]	0.55	0.55	0.48	0.48
DM, %	89.3	89.3	88.8	88.8
CP, %	16.2	16.4	17.3	17.5
ME, kcal/kg	3322	3324	3343	3345
NE, kcal/kg	2533	2534	2533	2534
Ca, %	0.77	0.77	0.77	0.77
P, %	0.61	0.61	0.62	0.62
Amino Acids, % SID				
Lys	1.03	1.22	1.03	1.22
Arg	0.93	0.93	0.93	0.93
His	0.46	0.46	0.46	0.46
Ile	0.64	0.64	0.64	0.64
Leu	1.23	1.23	1.33	1.33
Met+Cys	0.61	0.61	0.63	0.63
Phe+Tyr	1.04	1.04	1.17	1.17
Thr	0.72	0.72	0.72	0.72
Trp	0.28	0.28	0.28	0.28
Val	0.77	0.77	0.77	0.77

CP, crude protein; DM, dry matter; EAA-N, essential amino acid nitrogen; ME, metabolizable energy; NE, net energy; SID, standardized ileal digestible; TN, total nitrogen.

[1] Supplied per kilogram of complete feed: vitamin A, 4 000 IU; vitamin D, 0.019 mg; vitamin E, 15 IU; vitamin B12, 0.01 mg; menadione, 1.0 mg; thiamine, 0.50 mg; riboflavin, 2.0 mg; pyridoxine, 1.0 mg; niacin, 10 mg; pantothenate, 6 mg; folic acid, 0.25 mg; biotin, 0.05 mg; Cu, 7.5 mg; Fe, 50 mg; Mg, 20 mg; I, 0.50 mg; Zn, 50 mg, and Se, 0.15 mg.

[2] Nutrient content of diets based on estimated nutrient contents of feed ingredients according to NRC (2012).

[3] EAA-N:TN ratios of 0.55 (LP diets) and 0.48 (HP diets) reflect the amount of nitrogen in the diets coming from essential amino acids (EAA-N) and from the other components (TN), with the higher ratio having a larger contribution of nitrogen from EAA.

Table 5.2 Analyzed nutrient content (% as-fed basis) of experimental diets[1]

	High Ratio		**Low Ratio**	
Lys, % SID	**1.03**	**1.22**	**1.03**	**1.22**
DM, %	88.3 (89.3)	87.4 (89.3)	88.4 (88.8)	87.7 (88.8)
CP, %	16.9 (16.1)	16.5 (16.4)	17.2 (17.3)	17.5 (17.5)
Total Amino Acid, %				
Lys	1.12 (1.13)	1.16 (1.32)	1.05 (1.14)	1.31 (1.33)
Met	0.40 (0.43)	0.41 (0.43)	0.28 (0.43)	0.38 (0.43)
Met + Cys	0.68 (0.69)	0.68 (0.69)	0.48 (0.72)	0.63 (0.72)
Thr	0.67 (0.82)	0.67 (0.82)	0.70 (0.83)	0.71 (0.83)
Arg	0.94 (0.87)	0.91 (0.87)	0.97 (1.01)	0.97 (1.01)
Ile	0.73 (0.71)	0.70 (0.71)	0.73 (0.73)	0.75 (0.73)
Leu	1.41 (1.39)	1.34 (1.32)	1.46 (1.51)	1.52 (1.51)
Val	0.88 (0.87)	0.85 (0.87)	0.87 (0.87)	0.90 (0.87)
His	0.44 (0.51)	0.44 (0.51)	0.48 (0.52)	0.47 (0.52)
Phe	0.73 (0.72)	0.71 (0.72)	0.81 (0.81)	0.81 (0.81)

Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Met+Cys, methionine + cysteine; Phe, phenylalanine; SID, standardized ileal digestible; Thr, threonine; Val, valine.

[1] Analyzed total amino acids (AA) with calculated values in parentheses.

Table 5.3. Growth performance of pigs fed an LR or HR diet with increasing either 1.03 or 1.22 % SID lysine[1]

	Ratio		**Lysine**		**0.48**		**0.55**			***P*-value**		
Item	**0.48**	**0.55**	**1.03**	**1.22**	**1.03**	**1.22**	**1.03**	**1.22**	**SEM**	**Ratio**	**Lys**	**Ratio× Lys**
Body Weight, kg												
Day 0	19.72	19.57	19.63	19.66	19.63	19.81	19.62	19.51	0.24	0.36	0.85	0.40
Day 7	24.17	23.62	23.59	24.20	23.77	24.58	23.41	23.83	0.31	0.01	<0.01	0.37
Day 14	31.21	31.02	30.62	31.61	30.53	31.90	30.71	31.32	0.71	0.65	0.03	0.39
Day 21	39.55	39.16	38.74	39.97	38.80	40.31	38.68	39.63	0.58	0.35	<0.01	0.50
Day 28	47.03	46.79	45.98	47.85	46.03	48.03	45.93	47.66	0.76	0.66	<0.01	0.80
Average Daily Gain, kg/d												
Days 0-7	0.63	0.58	0.56	0.64	0.58	0.68	0.54	0.61	0.03	<0.01	<0.01	0.57
Days 8-14	1.03	1.06	1.01	1.08	0.99	1.08	1.04	1.09	0.04	0.27	0.02	0.53
Days 15-21	1.09	1.08	1.06	1.11	1.06	1.12	1.06	1.10	0.02	0.65	0.05	0.71
Days 22-28	1.13	1.17	1.11	1.19	1.10	1.16	1.13	1.22	0.02	0.03	<0.01	0.45
Days 0-28	0.98	0.99	0.95	1.02	0.95	1.02	0.96	1.03	0.01	0.31	<0.01	0.75
Average Daily Feed Intake, kg/d												
Days 0-7	1.15	1.12	1.12	1.15	1.14	1.17	1.10	1.14	0.05	0.02	0.06	0.73
Days 8-14	1.63	1.61	1.58	1.66	1.60	1.66	1.56	1.66	0.03	0.47	<0.01	0.38
Days 15-21	1.86	1.85	1.80	1.91	1.81	1.91	1.79	1.91	0.03	0.75	<0.01	0.71
Days 22-28	2.05	2.06	2.03	2.09	2.02	2.09	2.04	2.08	0.04	0.92	0.04	0.52
Days 0-28	1.69	1.68	1.65	1.72	1.66	1.73	1.64	1.71	0.02	0.42	<0.01	0.87
Gain:feed, kg/kg												
Days 0-7	0.56	0.53	0.52	0.57	0.53	0.59	0.50	0.55	0.02	<0.01	<0.01	0.82
Days 8-14	0.64	0.66	0.64	0.66	0.63	0.65	0.66	0.66	0.01	0.10	0.27	0.31
Days 15-21	0.58	0.58	0.58	0.57	0.58	0.58	0.59	0.57	0.01	0.93	0.49	0.42
Days 22-28	0.54	0.56	0.54	0.56	0.54	0.55	0.55	0.57	0.01	0.08	0.29	0.83
Days 0-28	0.59	0.59	0.59	0.60	0.59	0.60	0.59	0.59	0.01	0.78	0.13	0.63

ADFI; average daily feed intake; ADG, average daily gain; G:F,gain:feed; HR, high ratio (0.55); LR, low ratio (0.48); SID, standardized ileal digestible; SEM, standard error of the mean.

[1] Data are least-square means (*n*=12 pens/treatment).

Table 5.4. Apparent total tract digestibility of N and fecal N output in pigs fed an LR or HR diet with either 1.03 or 1.22 % SID lysine[1]

	Ratio		**Lysine**		**0.48**		**0.55**			***P*-value**		
Item	**0.48**	**0.55**	**1.03**	**1.22**	**1.03**	**1.22**	**1.03**	**1.22**	**SEM**	**Ratio**	**Lys**	**Ratio × Lys**
ATTD, %	80.7	81.1	80.5	81.3	81.9	79.6	79.2	83.1	0.76	0.48	0.21	<0.01
Fecal N, g/d	7.43	7.02	7.43	7.01	7.32	7.56	7.55	6.48	0.25	0.07	0.06	<0.01

ATTD, average total tract digestibility; HR, high essential amino acid-N:total N ratio (0.55); LR, low essential amino acid-N:total N ratio (0.48); N, nitrogen; SEM, standard error of the mean; SID, standardized ileal digestible.

[1] Data are least-square means (n=12 pens/treatment).

6.0 GENERAL DISCUSSION AND SUMMARY

Due to the rise in demand and cost of high-quality protein sources, it has become imperative for producers and nutritionists to implement production strategies to improve pork production while lowering its cost and negative environmental impact. High protein diets supply the pig with adequate nutrients to meet their requirements, however, protein and amino acids (AA) are generally oversupplied, resulting in decreased nitrogen (N) utilization efficiency for lean gain, and increased N excretion (Wang et al., 2018). Providing pigs with low protein (LP) diets supplemented with crystalline AA (CAA) is a current feeding strategy used to improve N utilization efficiency, lower N excretion into the environment, and reduce overall cost of the diet (Gloaguen et al., 2014). Although an effective strategy, feeding CAA-supplemented LP diets it assumes enough non-essential AA (NEAA) are being supplied and/or produced endogenously to meet the pig's requirements. However, this may not be the case, resulting in NEAA or N become limiting if the crude protein (CP) level is reduced by more than 4% units (specifically from 17.6% to 13.5% according to Gloaguen et al. 2014) (Křížová et al., 2001; Gloaguen et al., 2014; Mansilla et al., 2017a). The excess essential AA (EAA) may then be catabolized to obtain N for the synthesis of NEAA, resulting in the inefficient utilization of EAA for protein synthesis (Lenis et al., 1999). The consideration of NEAA or N requirements is important, as appropriately meeting these requirements would improve utilization efficiency of EAA for protein synthesis, improve NR, and lower N excretion.

The ideal protein concept has been used to formulate diets, assuming an ideal ratio of AA to maximize growth performance (Heger, 2003). However, the ideal protein concept does not consider NEAA requirements, although they supply almost half of the total dietary N required by the pig for the endogenous synthesis of NEAA (Heger, 2003). As a result, the application of the ideal protein concept to LP diets may not be appropriate as EAA requirements may change when NEAA are limiting (Wu and Li, 2022). The EAA-N to total N (TN) ratio (EAA-N:TN) considers the relationship between EAA and NEAA, on an N basis, which may be useful to determine appropriate requirements and supply of both EAA and NEAA (Wang and Fuller, 1989; Lenis et al., 1996; Heger et al., 1998; Heger, 2003). Heger et al. (1998) determined that when dietary EAA-N was held constant, and the TN fraction was altered, the ideal ratio to optimize NR is 0.48. Consequently, the studies presented in this thesis investigated the effects of feeding diets with either an optimal ratio (or LR), as defined by Heger et al. (1998) as 0.48, or an HR of 0.55, which is considered deficient in dietary N, on the Lys requirement and Lys utilization for NR and growth in pigs. It was hypothesized that pigs fed the HR diets would require more EAA (i.e., Lys; Chapter 4), and that pigs fed either LR diets with standard Lys or HR diets with increased Lys would have improved growth performance (Chapter 5).

In Chapter 4, an N-balance study was conducted to determine the effect of the EAA-N:TN ratio on Lys requirement for NR in growing pigs. In this study, pigs were fed diets formulated to have either a LR (0.48) or a HR (0.55) with five graded levels of Lys, at 0.82, 0.92, 1.02, 1.12, and 1.22 % SID Lys, representing 80, 90, 100, 110, and 120% NRC (2012) recommended requirements. These diets were fed to the pigs over a period of 11 d, with a 7-d dietary and environmental adaptation followed by a 4-d urine and fecal collection period. Breakpoint analyses estimated a Lys requirement of 1.21% SID at an NR of 17.8 g/d in pigs fed the HR diets. There was no breakpoint achieved for pigs fed the LR diets. Pigs fed the LR diets appeared to have better N utilization efficiency compared to pigs fed the HR diets, as indicated by increased NR, and PD, and decreased PUN. In addition, pigs fed the HR diets had increased plasma concentrations of the EAA Lys, Met, Trp, and Val, which may be indicative of decreased utilization for protein synthesis. Similarly, the plasma concentrations of NEAA Leu, Asp, Gly, and Ser were decreased in pigs fed the HR diets, which may be due to a low dietary supply, or insufficient endogenous synthesis. An increase in Lys resulted in a decrease in plasma concentrations of EAA His, Ile, Phe, Thr, Tyr, and Val, which may indicate an increase in protein synthesis with increasing Lys. Finally, increasing Lys also resulted in an increase in the plasma concentrations of NEAA Gln and Gly, and a decrease in Asn and Pro.

These results suggest that NEAA or N may become limiting in HR diets, but sufficient N was provided in LR diets. These results are supported by previous studies that determined that NEAA or N become limiting in LP diets (Gloaguen et al., 2014; Mansilla et al., 2017a). Furthermore, it indicates that current Lys requirements may not be adequate for maximizing growth potential, regardless of ratio. Similarly, the improved N efficiency observed in pigs fed the LR diets compared to the pigs fed the HR diets suggests that EAA are being utilized as an N source in pigs fed the HR diets (Heger et al., 1998; Millet et al., 2018a). The appropriateness of the optimal ratio of 0.48 is supported by the results of the current study as NR, PUN, and Lys efficiency are improved in pigs fed the LR diets compared to pigs fed the HR diets. The results of the present study indicate that NEAA or N may be limiting in HR diets, and that more Lys is required in LR diets to maximize NR. Overall, these results suggest that NEAA and N requirements should be considered and that the EAA-N:TN ratio may be an appropriate value to estimate sufficiency of dietary N supply.

A follow-up study was conducted to confirm the results of EAA-N:TN ratio and dietary Lys content on growth performance and N excretion (Chapter 5). It was hypothesized that growth performance and N excretion would be improved in pigs fed the LR diets with standard Lys content or in pigs fed HR diets with supplemental Lys. Growth performance measures (ADG, ADFI, G:F, and BW) were recorded weekly for 28 d and N digestibility was determined on d 14. The high and low EAA-N:TN ratios were the same as for Chapter 4, and Lys was included to either meet NRC (2012) requirement or the requirement determined previously in pigs fed HR dets. It was found that increasing dietary Lys content improved ADG and increased ADFI and BW, regardless of EAA-N:TN ratio. There was an observed increase in ADG in pigs fed the LR diets during week 1, whereas ADG was increased in pigs fed the HR diets during week 4. In addition, ADFI in pigs fed the LR diets during week 1 was increased in comparison to pigs fed the HR diets. These results suggest that there may be differences in nutritional requirements during the early and later growth stages, and the EAA-N:TN ratio and N supply may be of greater importance when maximizing growth potential. The growth performance study indicates that pigs fed the HR diets with 1.22% SID Lys had improved digestibility and lower N output, indicating a decrease in N excretion (Chapter 5). The decrease in N output is consistent with results obtained by Heger et al. (1998) and Lenis et al. (1999). The pigs fed the LR with 100% NRC (2012) requirements at 1.03% SID Lys had the next highest ATTD of N value, which would be consistent with the theory of 0.48 being the optimal ratio.

Although the results presented in this thesis indicate a higher Lys requirement than what is currently recommended, regardless of ratio, there was an observed increase in N utilization in pigs fed the optimal (or LR) diets in the N-balance, which is consistent with previous research (Heger et al., 1998). Consequently, increasing dietary Lys resulted in improved growth performance in pigs fed the HR diets (Chapter 5). Furthermore, EAA-N:TN ratios should be considered in diet formulations, as diets with a high EAA-N:TN may be limiting in NEAA or N.

A limitation of this research is the dietary protein content, which was greater than what has been examined in other studies investigating the effects of feeding LP diets (Heger et al., 1998; Otto et al., 2003; Gloaguen et al., 2014; Mansilla et al., 2017a). Although the dietary protein content exceeded the recommended minimum required to not compromise growth performance (i.e., 12-13% CP), it is still lower than traditional HP diets (i.e., 19-20% CP). In the present study, the dietary protein content could not be reduced further without compromising the desired ratios. In addition, although the diets are mostly practical, the use of soy protein concentrate is not common, resulting in the question of whether the diets used in the present study are truly practical for commercial application. Determining requirements utilizing the N balance method has limitations, including assumptions that all N retained is utilized for lean gain (protein deposition) and that N losses (i.e., nitrogen volatilization) are minimal. This may result in an overestimation of NR and, consequently, underestimation of requirements (Elango et al., 2012). Furthermore, the utilization of Lys as the test EAA was due to it being the first limiting AA with regards to growth, and it not being utilized primarily by the gut for energy (Li et al., 2008). However, it was not known whether Lys would be preferentially catabolized for N, as it does not undergo transamination, resulting in other EAA being limiting rather than Lys. Consequently, it may be of benefit to investigate the effects of the EAA-N:TN ratio in LP diets with an EAA more heavily involved in N metabolism, such as Ile, in future studies. Another limitation was the lack of an additional SID Lys content in the growth performance study (Chapter 5). The SID Lys levels used in that study were based on the breakpoint values achieved with the HR diets in the N-balance study (Chapter 4) and were within the linear portion of the response curve. Furthermore, no interactive effects of ratio and Lys level were observed, indicating a third Lys level may have been beneficial. By utilizing a third higher SID Lys content, the pigs fed the LR diets may have had improved observed growth performance compared to those fed the HR diets. The N-balance study (Chapter 4) consisted of 80 barrows, rather than a mix of gilts and barrows, due to N-balance collection being easier with barrows. It is possible that sex differences may arise, due to their differing growth patterns. For instance, gilts tend to have improved feed efficiency, whereas barrows have increased feed intake and deposit more fat during the later growing/finishing period (Cromwell et al., 1993). In addition, it was observed that an increase in dietary protein or Lys content improve lean gain in gilts, but not barrows (Cromwell et al., 1993). Similarly, Warnants et al. (2008) determined that 40-70 kg gilts required a higher SID Lys content to improve performance when compared to barrows. Consequently, although the barrows acquired in Chapter 4 were much younger than the ones investigated in the experiments described above, it is evident that gilts may require more Lys to achieve maximum NR in the later growing stages than what was determined with the barrows in Chapter 4. Finally, it is possible that Lys requirements may differ between populations, and more or less may be required based on the genetic lines of those populations as well as the estimated maximum PD observed with the NRC (2012) model. Based on the limitations and the results of the present studies, future research considering protein level, EAA-N:TN ratio, other test EAA specific to N metabolism, pig sex, genotype, and population, should be conducted to quantify an appropriate Lys level to maximize growth potential in growing pigs.

Overall, the research presented in this thesis demonstrates that current Lys requirements (NRC, 2012) may not be sufficient to maximize growth in 20-50 kg growing pigs. In addition, the consideration of the EAA-N:TN ratio may be beneficial to diet formulation as it considers the amount of N available for utilization and may improve NR and decrease N excretion. By improving N utilization and retention, diets may be better formulated to match the animal's requirements for protein and AA, which would reduce costs for the producer and reduce N excretion into the environment. Further research should focus on defining an appropriate EAA-N:TN ratio to optimize production performance and limit N excretion into the environment. In addition, it would be beneficial to explore the effects of the EAA-N:TN ratio on the requirements for other EAA, such as those more heavily involved in N metabolism. Future studies in this area would be beneficial to the pork industry as they may lower feed costs, improve production performance, and minimize environmental impact.

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