2018

Efficiency of Utilizing Standardized Ileal Digestible Lys and Thr for Whole Body protein Retention in Pregnant Gilts during Early, Mid and Late Gestation

Ron Aldwin Sapin Navales
South Dakota State University

Recommended Citation

Navales, Ron Aldwin Sapin, "Efficiency of Utilizing Standardized Ileal Digestible Lys and Thr for Whole Body protein Retention in Pregnant Gilts during Early, Mid and Late Gestation" (2018). *Electronic Theses and Dissertations*. 2649.
https://openprairie.sdstate.edu/etd/2649

EFFICIENCY OF UTILIZING STANDARDIZED ILEAL DIGESTIBLE LYS AND THR FOR WHOLE BODY PROTEIN RETENTION IN PREGNANT GILTS DURING EARLY, MID AND LATE GESTATION

BY

RON ALDWIN SAPIN NAVALES

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2018

EFFICIENCY OF UTILIZING STANDARDIZED ILEAL DIGESTIBLE LYS AND THR FOR WHOLE BODY PROTEIN RETENTION IN PREGNANT GILTS DURING EARLY, MID AND LATE GESTATION

RON ALDWIN SAPIN NAVALES

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Crystal Levesque, Ph.D.
Thesis Advisor — Date

Joseph Cassady, Ph.D.
Head, Department of Animal Science — Date

Dean, Graduate School — Date

Ang lahat ng ito ay iniaalay ko Sa'yo Hesu-Kristo, hindi man karapatdapat ang makasalanan na tulad ko, ay binigyan mo ako ng pambihirang pagkakataon.

Mula sa Diyos Ama,
sa pamamagitan ng Diyos Anak,
at sa kapangyarihan ng Diyos Espirito Santo

-Ron Aldwin Sapin Navales
Republic of the Philippines

ACKNOWLEDGMENTS

As the author completes his MSc thesis, it is a great pleasure for him to express his sincerest gratitude and appreciation to the following person who made this all possible.

His major and thesis advisor, **Dr. Crystal Levesque**, for her support and patience in giving invaluable advice from the day they drafted the research protocol until they finished this thesis manuscript. He would like to thank her for accepting him in her laboratory and research group. He also would like to thank **Dr. Robert Thaler**, his graduate committee member and his “extension” advisor, for his assistance, encouragement and answering his extension-related questions. The author will never forget how they became a great part of his journey of fulfilling his dream to become a swine nutritionist.

The **Foundation for Food and Agriculture Research: New Innovator Award**, **ADM Animal Nutrition**, **Ajinomoto North America** and **Evonik Nutrition & Care GmbH** for funding this research.

He also would like to acknowledge **Dr. Garry Hatfield** for his advice on statistical analyses and to **Drs. Tofuko Woyengo**, **Ryan Samuel**, **Derek Brake** and **Robbi Pritchard** for their recommendations and for answering his research-related questions.

Studying in SDSU will not be possible for him without the help of **Drs. Rommel Sulabo** and **Amado Angeles** for their recommendation letter and for connecting him to SDSU Animal Science Department. Their never-ending support for young Filipino animal nutritionist is essential for the continuous growth of the Philippine livestock and poultry industry. Equally, he is thankful to **Dr. Arthus Roxas** of **Ariela Marketing Company, Inc.** for his moral and financial support.

Of course he is grateful to **Deon Simon** and **Jason Griffin**, for their assistance in laboratory analyses, to **Dr. Emily Miller** for assisting him in catheter insertion, and to **Cameron "Kuya" Pewe** and **Joe Wollbrink** for their assistance in animal works.

The author will not forget all the graduate and undergraduate students who helped him throughout his graduate study program namely: **Jamie Ortman**, **Samantha Welu-Tauer**, **Jake Koepke**, **Alicia Levesque**, **Dr. Agatha Ampaire**, **Jungwok Lee**, **Emily Scholtz**, **Casey Zangaro**, **Molly Renken**, **Necie Abelilla**, **Alejandro Casella**, **Christian Ramirez**, **Talia Everding**, **Lily Hernandez** and **Katelyn Zeamer**. They assisted him not only with his research but also in adjusting in South Dakota. He will always consider them as his friends.

He also would like to thank his extended family in the US: **Matthew, Janet and Samuel Miller**, **Maria Palma-Hall**, **Amelia Toutges**, **Nic** and **Kimberly Cayetano**, **Camille Ortega**, **Manny Tubandt**, **Deepak Joshi** and **Prakriti Sharma**. They may not be related by blood but being with them was like home.

To his family in the Philippines, to his mother, **Flora Navales**, his sisters, **Verna** and **Mariflor Navales,** and to his late father **Mario Navales** for being his source of inspiration. He was able to get through the physical, mental and emotional stresses because of them. Every time he asked himself if he made the right decision, he always remembers that his dreams are for his family.

Above all, the author would like to give the highest recognition to the **God the Father** who created him, to **God the Son** who saved him, and to **God the Holy Spirit** who blesses him. For the author, MSc is not and will never be Master of Science, for him it means *“**M**ula **S**a’Yo **C**risto”* (From You, Christ) as his degree came from Christ and will always be for the greater glory of Christ’ name.

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ABBREVIATIONS

AA	amino acid(s)
ADG	average daily gain
ATPase	adenosine triphosphatase
BW	body weight
$BW^{0.75}$	metabolic body weight
°C	degree centigrade
Cfat	crude fat
CP	crude protein
Cys	Cysteine
d	day(s)
DE	digestible energy
DE_{maint}	digestible energy for maintenance
DMI	dry matter intake
g	gram
K	Potassium

kg	kilogram
kcal	kilocalories
$k_{SID}Lys$	efficiency of utilizing SID Lys whole body protein retention
$k_{SID}Thr$	efficiency of utilizing SID Thr whole body protein retention
Ld	lipid deposition
Leu	Leucine
Lys	Lysine
m	meter
Mcal	megacalories
ME	metabolizable energy
ME_{maint}	metabolizable energy for maintenance
Met	Methionine
mg	milligram
mL	milliliters
N	Nitrogen
Na	Sodium
NE	net energy

NRC	National Research Council
Pd	protein deposition
SAS	Statistical Analysis System
SEM	standard error of the mean
SID	standardized ileal digestible/ity
Thr	Threonine
Trp	Tryptophan
TID	true ileal digestible/ity
USDA	United States Department of Agriculture
USPCE	US Pork Center of Excellence
Val	Valine

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ABSTRACT

EFFICIENCY OF UTILIZING STANDARDIZED ILEAL DIGESTIBLE LYS AND THR FOR WHOLE BODY PROTEIN RETENTION IN PREGNANT GILTS DURING EARLY, MID AND LATE GESTATION

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In pregnant pigs, amino acid (AA) requirements represent the sum of those required for maintenance functions, protein retention and efficiency of utilizing AA intake for the aforementioned body processes. The NRC (2012) model assumed AA efficiency is constant across period of gestation; however this is not reflective of the changes in metabolic demand during gestation. Therefore, two experiments were conducted to evaluate the efficiency of utilizing SID Lys and Thr for whole body protein retention (k_{SID}Lys and k_{SID}Thr) in pregnant gilts during early, mid and late gestation. Three 12 d N-balance studies were conducted to represent different periods of gestation. Graded levels of Lys and Thr moderately below the NRC (2012) requirements were used to estimate the AA efficiency within balance periods. Lysine and Thr efficiency using regression analysis could not be determined for early and mid-gestation because of the lack of response in Lys and Thr retention to increasing SID Lys and Thr intake, respectively, which reflects an oversupply of the respective test AA. At the lowest SID Lys and Thr intake, Lys and Thr efficiency were 0.49 and 0.32 for early gestation and 0.61 and 0.52 for mid-gestation, respectively. In contrast, k_{SID}Lys and k_{SID}Thr in late gestation were determined to be 0.54, which is slightly higher than the current NRC

(2012) estimate of 0.49 and 0.53 for Lys and Thr, respectively. Evidences from our current study suggest that k_{SID}Lys and k_{SID}Thr are not constant throughout gestation and therefore not reflective of the changes in metabolic demand of pregnant pigs during pregnancy. Also, the lack of response to dietary SID Lys and Thr levels suggest that SID Lys and Thr requirements of pregnant gilts are lower (i.e. <10 g SID Lys/d and <6 g SID Thr/d) than the current NRC (2012) recommendation of 11 g SID Lys and 8 g SID Thr/d from d 0 to 90 of gestation; whereas the requirements for SID Lys and Thr during late gestation (>90 d) is reasonably represented in NRC (2012) at 17 and 12 g/d, respectively. Our current research is important for the refinement of the AA requirement model for gestating pigs to ensure diet optimization, nutrient excretion management and improvement of overall farm efficiency.

CHAPTER 1

Lysine and Threonine Requirements of Pregnant Gilts: Literature Review

1.1 Introduction

Hog production in the US is expected to increase 5% in 2018 from 11.61 million metric tons in 2017 driven by higher hog slaughter and gains in carcass weight. While strong domestic and export demand boosted hog prices in 2017; rising supplies, along with simultaneous growth in production among exporters and reduced demand from top importers, are forecasted to drive live hog prices down 9% for the year (USDA, 2018). Production and economic losses can be mitigated by strong demand for pork. United States exports are expected to rise nearly 5%, but the forecast is marginally lowered due to the impact of China’s imposition of tariffs on US pork. Similarly, economic losses can be reduced through low production cost. The estimated returns in a farrow to finish operation presented in Iowa State University’s Estimated Livestock Return series showed that feed cost accounts for 62% of the total production cost (ISU, 2018); therefore, lower feed costs can have a significant influence in reducing overall production cost.

Precision feeding offers opportunity for improving swine herd efficiency and reducing overall production cost. In precision feeding, nutrients are supplied sufficient to meet animal requirements with minimal excess and relies on accurate mathematical models to estimate nutrient requirements. A number of studies had been made to estimate the nutrient requirements of growing pigs, but there are limited empirical studies for the breeding herd where feed cost constitute about 12% of the cost of producing a market hog (Aherne, 2006; Calud and Tamisin, 2014). In addition to profitability, precision feeding in the breeding herd can positively impact sow productivity and longevity as early culling are often related to extreme variations in body reserves (Dourmad et al., 1994; Dourmad et al., 2008).

The recent edition of NRC (2012) Swine Nutrient Requirements is an improved model for estimating nutrient requirements of pregnant pig that considers the change in metabolic demand from early to late gestation. However, the model is based on a paucity of data in pregnant gilts and sows and assumptions derived from empirical studies in growing-finishing pigs. The lack of empirical data includes amino acid (AA) requirements, the second highest contributor to formula cost following energy and where more requirement research has been conducted than any other class of nutrients. Given this gap, it is important to review the available information on AA requirements of gestating sows. This literature review focuses on standardized ileal digestible (SID) lysine (Lys) and threonine (Thr) requirements of pregnant gilts during early, mid and late gestation, primarily because these are the two most limiting AA in a corn-soybean meal-fed pigs.

1.2 Objectives

The objective of this literature review is to define the existing models for estimating amino acid requirements of gestating pigs, factors that influence protein uptake and retention and the dynamics of amino acid requirements during early, mid and late gestation. In addition, the practical significance of the model for decision makers and pork producers will be presented.

1.3. Model for estimating AA requirements of gestating sows

Mathematical models, based on the factorial approach, have been used to estimate the nutrient requirements of different classes of swine. For reproducing pigs, this is important as nutritional supply must be adapted to maintain body reserves in optimal condition throughout their productive life and optimize reproductive performance (Dourmad et al., 2008). Nutrient utilization in gestating sows, as described in Figure 1-1, suggests that priority is given to maintenance requirements and gravid uterine growth (fetus, fluids and membranes and empty uterus). Excess nutrients constitute the sow body reserves (i.e. maternal body lipid and protein). When nutrient intake is insufficient, body reserves are mobilized to support maintenance needs and gain of conceptus.

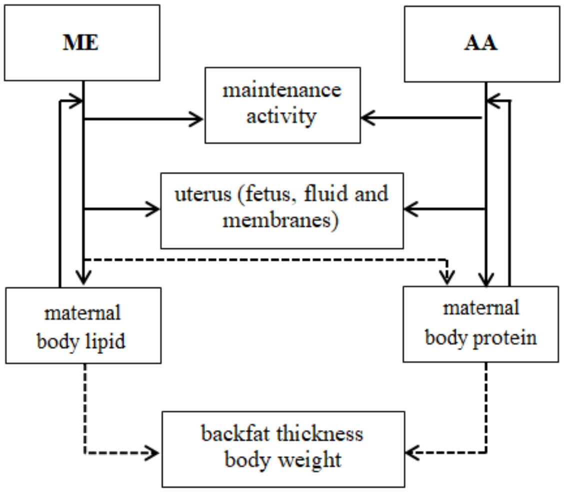

Figure 1-1. Nutrient utilization in pregnant sow [adapted from Dourmad et al. (2008)]

In the recent NRC (2012) gestating sow nutrient requirement model, energy intake and animal performance (i.e. sow body weight (BW) at breeding, parity, anticipated litter size, and anticipated piglet birth weight) are defined as model inputs. Energy is partitioned to maintenance requirements, energy retention in products of conception, and maternal body protein deposition (Pd) and lipid deposition (Ld). Change in maternal BW is predicted from changes in body protein and lipid mass; whereas weight gain of conceptus is represented as a function of anticipated litter size at birth, mean piglet birth weight and days into gestation (NRC, 2012; de Lange, 2013). Nutrient (AA, total N, Ca and P) requirements to support metabolism and the observed animal performance are then generated. Specifically, for AA and total N, requirements represent the sum of those required for maintenance functions and protein retention.

1.3.1. Amino acid requirements for maintenance

NRC (2012) described the maintenance requirements for AA in gestating sows consistent with Moughan (1999), where it includes the basal endogenous intestinal AA losses and skin and hair AA losses. Basal endogenous losses which are related to dry matter intake (DMI) account for AA secretions into the intestinal tract that are not reabsorbed by the pig. Basal total intestinal endogenous AA losses are taken as 110% of basal ileal endogenous losses to account for the contribution of large intestine to basal total intestinal endogenous AA losses (Moughan, 1999). For gestating pigs, Lys loss of endogenous origin is equivalent to 0.522 g/kg DMI and is based on the earlier studies of Stein et al. (1999) in restricted fed sows. Whereas, Thr loss was calculated from the ideal AA profile (AA content relative to Lys) generated from ileal cannulation studies in growing pigs reported in literature and is equivalent to 0.757 g/kg DMI (NRC, 2012). Estimate of basal endogenous intestinal Thr loss reported in NRC (2012) is higher than the values obtained by Stein et al. (1999) for restricted and *ad libitum* fed gestating sows at 0.606 and 0.508 g/kg DMI, respectively. Recent studies in growing pigs report endogenous intestinal losses ranging from 0.430 to 0.490 g/kg DMI for Lys and 0.420 to

0.550 g/kg DMI for Thr (Stein et al., 2005; Zhai and Adeola, 2011; Xue et al., 2014; Adeola et al., 2016). Daily AA losses via skin and hair are estimated as a function of $BW^{0.75}$ and are equivalent to 4.50 and 3.35 mg/kg $BW^{0.75}$ for Lys and Thr, respectively (Whittemore et al., 2001; van Milgen et al., 2008). The current NRC (2012) model provides a more mechanistic estimate of Lys and Thr requirements for maintenance function. This is in contrast to the NRC (1998) that uses fixed values of 36.00 and 54.36 mg/kg $BW^{0.75}$, respectively and are determined based on N-balance studies in growing pigs. The NRC (2012) values are also lower than the observations of Samuel et al. (2008) for Lys (49 mg/kg $BW^{0.75}$) and of Moehn et al. (2011) for Thr (98 mg/kg $BW^{0.75}$) using the indicator AA oxidation technique in adult sows. Moehn et al. (2011) noted that N-balance tends to underestimate maintenance requirements for AA.

1.3.2. Amino acid requirements for protein retention

Amino acid requirements for protein retention predicted by the NRC (2012) model are based on crude protein (CP) mass and AA composition of six protein pools: 4 pregnancy-associated protein pool (fetus, placenta plus fluids, uterus and mammary tissues) and 2 maternal-associated protein pool (time-dependent and energy intake-dependent maternal Pd).

Protein content of fetal tissue is estimated using natural logarithm as a function of days into gestation and anticipated litter size at farrowing [Eq. 8-56, NRC (2012)]. Crude protein mass in placenta plus fluids is calculated based on similar inputs but using Michaelis-Menten kinetics function [Eq. 8-57, NRC (2012)]. Calculated protein contents of fetal tissue and placenta plus fluids are then corrected for mean piglet birthweight that is based on a ratio between actual litter weight at birth and the anticipated litter birthweight [Eq. 8-58, NRC (2012)]. Protein contents of non-gravid uterine and mammary tissues are also calculated using natural logarithm but only consider days into gestation [Eq. 8-59 and 8-60, NRC (2012)]. The aforementioned equations assume that energy intake does not impact growth of conceptus, unless under severe energy intake restriction and the assumption has been demonstrated in number of studies. Jin et al. (2016) evaluated 4 energy intake levels (i.e. 6.2, 6.4, 6.6 and 6.8 Mcal/d) in pregnant gilts from breeding to d 110 of gestation and reported a non-significant difference in total born and birth weight. Conversely, Noblet et al. (1985), using comparative slaughter technique, reported a decrease in pregnancy associated Pd when pregnant sows were given 4.78 versus 7.17 Mcal ME per d. A more severe reduction in energy intake (2.2 versus 8.0 Mcal DE per d) in pregnant gilts during the entire gestation resulted in reduced piglet birth weight [(Bazer et al., 1968) as cited by (Ji et al., 2017)].

Energy intake-dependent maternal Pd is estimated relative to ME intake above maintenance ME requirement at breeding [Eq. 8-62, NRC (2012)]. The relationship is assumed to be linear and constant across period of gestation (NRC, 2012). The positive linear relation is supported by the findings of Miller et al. (2016) and Dourmad et al. (1996) for pregnant gilts; however, Miller et al. (2016) reported decreasing maternal Pd with day of gestation in gilts. The estimate of energy intake-dependent maternal Pd also uses a coefficient [Eq. 8-63, NRC (2012)] to account for the age of the sow. The coefficient declines from parity 1 to 4 and becomes zero at parity 5 when the sow effectively stops growing. Residual Pd that is not associated with energy intake-dependent maternal Pd or reproductive tissues is attributed to time-dependent maternal Pd (NRC, 2012). Protein gain in time-dependent maternal Pd only occurs during the first part of gestation (i.e. until d 56). Moehn and Ball (2013) speculated that time-dependent maternal Pd can be explained by regaining maternal tissue lost from previous lactation.

A simulation of predicted total protein gain (g/d) of a pregnant gilt weighing 140 kg at breeding, consuming 2.2 kg/d feed (3.3 Mcal/kg ME), with anticipated litter size of 12.5, and anticipated piglet birth weight of 1.4 kg based on the NRC (2012) model is shown in Figure 1-2.

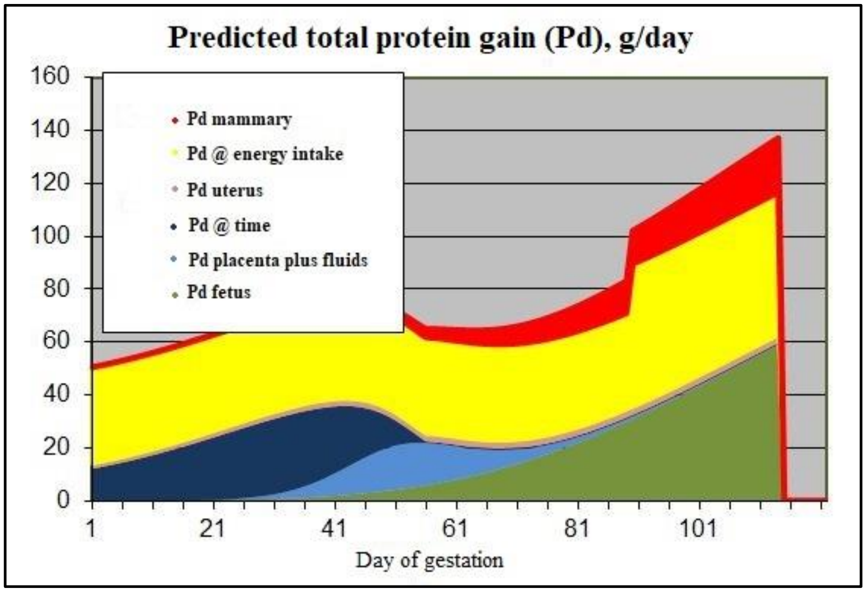

Figure 1-2. Predicted total protein gain (g/d) of pregnant gilt weighing 140 kg at breeding; consuming 2.2 kg/d feed (3.3 Mcal/kg ME); with anticipated litter size of 12.5; and anticipated piglet birth weight of 1.4 kg [adapted from NRC (2012)]

Amino acid composition of gestation protein pools are based on published data and empirical studies. Per 100 g CP, the Lys content of maternal, fetal, uterine, placental and mammary tissues are: 6.74, 4.99, 6.92, 6.39 and 6.55 g, respectively (Wu et al., 1999; NRC, 2012). Other essential amino acids are estimated relative to Lys. For Thr, this corresponds to 3.71, 2.79, 4.22, 4.22 and 5.24 g/100 g CP. A recent study evaluated the

AA compositions of fetal pig during development (d 45 to 114) and found that fetus contains 6.7 to 5.6 and 2.7 to 1.6 g Lys and Thr, respectively per 100 g AA (Hill and Mahan, 2016).

The NRC (2012) model provides a more detailed estimation of AA requirements for protein retention than the previous version (NRC, 1998) where AA requirements were estimated from total N retention and AA composition of tissue accretion based on growing-finishing pigs. Total N retention is the sum of maternal N retention and N retained in the products of conception. The former is estimated from gestation weight gain, whereas the latter is estimated from the expected number of pigs born. The Lys and Thr required to support one g of N retention are 0.807 and 0.484 g, respectively.

1.3.3. Efficiency of Amino Acid Utilization

In addition to maintenance functions and protein retention, the NRC (2012) model also considers the minimum and inevitable AA catabolism as a determinant in the calculation of total SID AA requirements of pregnant sows. This determinant is estimated from the inefficiency of utilizing SID Lys and Thr intake for various body functions at 0.25 and 0.19, respectively. The post-absorptive efficiencies of 0.75 and 0.81 for SID Lys and Thr are derived from observations on serial slaughter studies in growing pigs (30 to 70 kg BW).

The efficiency estimate is applied to maintenance functions to account for the minimum contribution of Lys and Thr to urinary N excretion. For protein retention, the base efficiency values of 0.75 and 0.81 for SID Lys and Thr are reduced to 0.49 and 0.53, respectively to account for between-animal variability and match the model-predicted with observed requirements from empirical studies. When NRC (2012) adjusted the model-predicted requirements to match the observed requirements, protein retention and AA utilization between d 90 and 114 of gestation were considered because during late gestation sow performance is most sensitive to AA intake.

In contrast to growing pigs, the marginal efficiency of utilizing SID Lys and Thr intake above maintenance in gestating pigs are not corrected for BW and performance potential. Similarly, the efficiency estimates are assumed to be identical across gestation protein pools, days of gestation and parities (NRC, 2012).

The Inraporc model (Dourmad et al., 2008) on the other hand estimates the efficiency of Lys utilization for protein retention at 0.65 (van Milgen and Dourmad, 2015). Potential limitations of other AA are derived from the ideal protein profile for gestation and AA composition of body protein and components of maintenance. As opposed to NRC (2012), the Inraporc model does not account for between-animal variability in the estimation of total SID AA requirements. Authors of the Inraporc model; however, suggest to increase the model-determined AA requirements by 10% in diet formulation (van Milgen and Dourmad, 2015).

1.3.4. Summary of Model Assumptions

Empirical studies on nutrient requirements are necessary for model development and testing. However, unlike the abundance of research in growing-finishing pigs, limited empirical nutrient requirements studies are available for pregnant pigs, thus assumptions are made in model development. For AA, these assumptions relate to the response of protein retention to energy intake and estimation of efficiency of utilizing SID AA intake for various body functions. In the model, it is assumed that energy intake-dependent maternal Pd is linearly related to ME intake above maintenance requirement and that the response [i.e. slope in Eq. 8-62, NRC (2012)] is identical across period of gestation but changes with parity. Moreover, energy intake and growth of conceptus is assumed to be independent unless under severe energy restriction (NRC, 2012). The relationship of protein retention and energy intake have been demonstrated in the earlier studies of King and Brown (1993) and Miller et al. (2016).

Correspondingly, NRC (2012) assumes that the efficiency of utilizing SID AA intake for maintenance functions and protein retention is constant across days of gestation. In contrast, Miller et al. (2017) revealed a quadratic increase in efficiency of Lys retention with day of gestation in second and third parity sows. However, as the study of Miller et al. (2017) is focused on the impact of energy intake to protein retention, a single diet oversupplied with all AA to meet requirements at d 90 to 114 of gestation (i.e. 0.82% SID Lys) was used. This implies that in the study of Miller et al. (2017) the excess SID Lys (and other AA) is higher during early than late gestation which can affect the efficiency response; thus direct application of Miller et al. (2017) to AA efficiency is limited.

1.4. Factors that influence protein retention in pregnant sows

Total SID AA requirements of pregnant sows are primarily determined by protein retention [estimated as N retention X 6.25, NRC (2012)]; thus, it is important to know how AA are digested and absorbed and the factors that affect amino acid utilization for protein retention.

1.4.1 Review of amino acid digestion and absorption

Digestion of protein, as described by Krehbiel and Matthews (2003) and Yen (2001), is initiated in the stomach. Parietal cells secrete HCl which denatures dietary protein and converts pepsinogen to pepsin promoting proteolysis of protein to large polypeptides. The pre-digestion increases the susceptibility of peptide molecules to hydrolysis by proteolytic enzymes in the small intestine. Amino acids and peptides are also good stimuli for the release of hormones that stimulate pancreatic enzyme secretion. In the duodenum, polypeptides are broken down further by trypsin, chymotrypsin, elastase and carboxypeptidases A and B. Inactive trypsinogen is converted to active trypsin by the removal of N-terminal peptide and the reaction is catalyzed by enterokinase. Trypsin then activates the other zymogens. The products of pancreatic digestion are approximately 60% oligopeptides (i.e. up to 6 AA residues) and 40% free AA. The final stage of protein digestion in the small intestine is mediated by brush border and cytoplasmic peptidases in the enterocytes. Oligopeptides with 3 or more AA residues are hydrolyzed by brush border peptidases (Step 1 in Figure 1-3); whereas tri- and dipeptides are either broken down by brush border and cytoplasmic peptidases or absorbed intact and transported into the circulation.

Absorption of digested protein (i.e. small peptides or free AA, in the lumen or mucosa) is facilitated by several transport mechanisms (Yen, 2001) and is summarized in Figure 1-3 (Steps 2 – 8). Peptides are absorbed across brush border membrane by PepT1 (2) and either transported intact across the basolateral membrane by a H^+-independent transport activity (3) or hydrolyzed to free AA by intracellular peptidases (4). Free AA’s in the lumen are transported to the brush border membrane via Na^+-dependent and independent AA transporters (5). These free AA’s plus those from hydrolyzed peptides

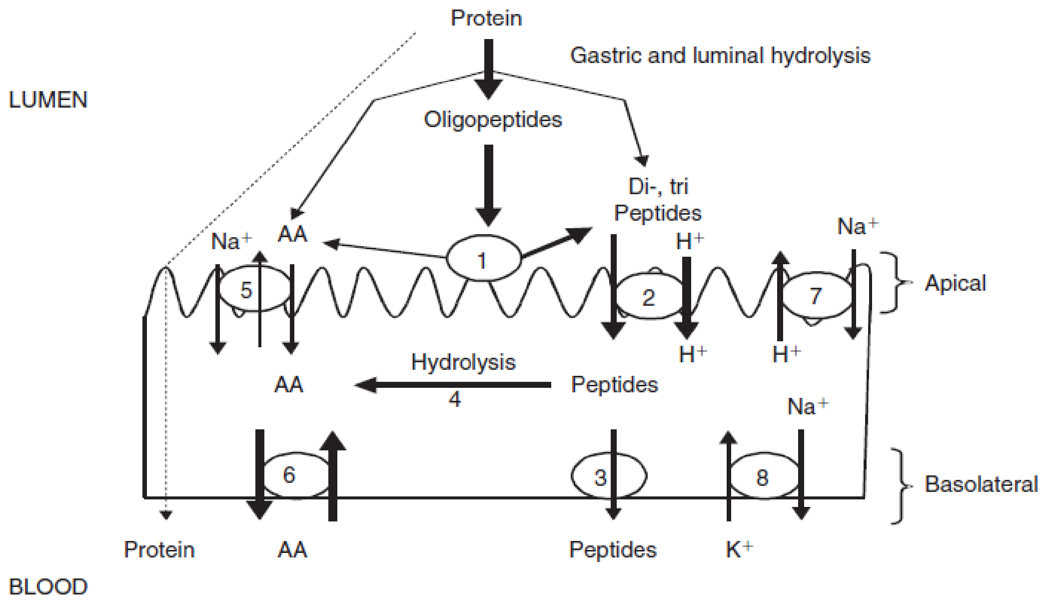

Figure 1-3. Absorption of digested protein (i.e. small peptides or free AA, in the lumen or mucosa) by enterocytes [adapted from Krehbiel and Matthews (2003)]

cross the basolateral membrane by a complement of Na^+-independent and AA exchanger transport proteins (6). Apical Na^+/H^+ exchanger (7) and basolateral Na^+/K^+ ATPase (8) help re-establish the extra- and intracellular H^+ gradient.

Once absorbed, AA are either catabolized or incorporated into protein. Pettigrew and Yang (1997) summarized that protein accretion are limited by three factors: animal potential, energy intake, or AA intake. Similarly, van Milgen and Dourmad (2015) and Kim et al. (2009) noted the importance of ideal amino acid balance in gestating sows for efficient AA utilization for protein retention.

1.4.2. Protein retention and animal potential

Gestating sows have a high potential protein accretion rate that varies with genetic strain and age (Pettigrew and Yang, 1997). The dynamics of nitrogen retention (g/d) in pregnant gilts across period of gestation have been reported in several studies and are presented in Table 1-1. Nitrogen retention increases as pregnancy progresses and this is attributed to the growth of products of conception and is consistent regardless of parity (Moehn and Ball, 2013). McPherson et al. (2004) observed cubic and quadratic responses of fetal weight and protein, respectively, as gestation progressed. Fetal protein growth is accelerated after d 69 of gestation from 0.25 to 4.63 g/d. A recent study of Hill and Mahan (2016) showed that the quantitative increase in AA occurred sharply after d 80 of gestation, particularly at d 100 to birth. Specifically, Lys and Thr content of fetus

Table 1-1. Dynamics of nitrogen retention (g/d) in pregnant gilts across period of gestation reported in several studies

Period of Gestation	N Intake g/d	N Retained, g/d	Method Used, Source
d 38 to 42	71.90	23.10	N-balance, Miller et al. (2016)
d 52 to 56	70.80	20.70	
d 66 to 70	74.10	23.10	
d 87 to 91	74.80	24.40	
d 108 to 112	73.20	27.20	
Early (d 40-50)	52.68	25.83	N-balance, Srichana (2006)
Mid (d 70-80)	53.20	26.44	
Late (d 90-100)	69.36	32.13	
Early (d <70)	39.14	6.37	Serial slaughter, Ji et al. (2005)
Late (d >70)		16.54	
Early (d 30-34)	52.60	11.90	N-balance, King and Brown (1993)
Mid (d 58-62))		12.80	
Late (d 86-90)		18.00	

increased by 17.5 and 17.2 fold from d 45 to d 80 of gestation (i.e. 0.085 and 0.034 g to1.494 and 0.587 g, respectively).

As protein retention increases with day of gestation, requirements for AA to support whole body protein gain increase. Simultaneously, the sow becomes more responsive to AA intake during late gestation due to the rapid increase in AA requirements (Pettigrew and Yang, 1997). The increase sensitivity to AA intake during late gestation explains why the NRC (2012) model estimated the AA efficiency of utilization for protein retention between d 90 and 114 of gestation and used it throughout gestation. The approach of using a consistent AA efficiency throughout gestation however is a deviation from the different marginal efficiency of Lys use calculated by Pettigrew and Yang (1997), using the data of King and Brown (1993), for early/mid and late gestation at 0.46 and 0.56, respectively. Although, King and Brown (1993) used eight experimental diets with increasing dietary AA level which is in contrast to Miller et al. (2017) that used a single diet; they use the same set of eight diets for early, mid and late gestation. Therefore, the direct application of Pettigrew and Yang (1997), using the data of King and Brown (1993), to calculate and compare AA efficiency between period of gestation is also limited.

In contrast to the increasing N retention to days of gestation, a summary provided by Moehn and Ball (2013) reported that whole body protein retention across parities 2 to 4 decreases. This can be explained by the largely similar fetal growth among parities and the reduced maternal growth as the sow ages. Lewis and Bunter (2013) observed a curvilinear growth in pregnant pigs through parity 5. Sow achieve the 90% of parity 5-BW by 22 months of age (i.e. parity 3).

1.4.3. Protein retention and energy intake

Weight gain during pregnancy is a result of maternal protein and lipid deposition, and conceptus gain (NRC, 2012). These anabolic processes (i.e. fat and protein biosynthesis) require energy. For protein, greater energy intake allows for more protein accretion (Pettigrew and Yang, 1997; Miller et al., 2016). The amount of accreted protein that can be supported per unit increase in ME is a measure of leanness. In pregnant sows, besides maternal protein gain, whole body protein accretion includes the product of conception. The latter is more responsive to incremental ME intake than the former. Pettigrew and Yang (1997), using the earlier study of Noblet et al. (1985) explained that the greater sensitivity of the product of conception to incremental ME intake is due to the high protein and very little fat composition of fetal tissues. Recent findings of McPherson et al. (2004) reported that the fetal carcass contains 58% CP and 13% crude fat (Cfat). Similarly, Miller et al. (2016) and (2017) compared 2 feeding levels (i.e. 1.87 versus 2.54 and 2.00 versus 2.75 kg/d) of a diet containing 3300 kcal/kg ME and reported a non-significant difference in pregnancy associated Pd but a significant decrease in maternal Pd for gilts and sows fed 1.87 and 2.0 kg/d, respectively. Results indicate a strong priority for developing the products of conception at the expense of maternal protein.

The positive linear relationship of N retention and ME intake across period of gestation has been shown in the studies of King and Brown (1993); Dourmad et al. (1996); Miller et al. (2016). A linear-plateau response was not demonstrated by these studies as the energy intake levels used were below 3 times maintenance. Campbell et al. (1985), as cited by Dourmad et al. (1996), observed the maximum N retention in finishing pigs at 3 times ME for maintenance (ME_{maint}). Chu et al. (2012) found that the optimum DE intake for maximum lean deposition in 79 to 106 kg pig is 9.84 Mcal/d [ME is 92-98% DE, NRC (1998)]. Using the estimated ME_{maint} requirement of 197 kcal/kg $BW^{0.60}$ for growing pigs (NRC, 2012), it can be calculated that the later findings of Chu et al. (2012) agrees with Campbell et al. (1985) at 3.1 times ME_{maint}. This implies that under practical conditions, the energy feeding levels for pregnant gilts and sows are below the level required for maximum N retention (King and Brown, 1993; Dourmad et al., 1996).

In contrast to N retention, there are few studies evaluating the relationship of energy intake and AA composition of pigs. In a study conducted by Bikker et al. (1994), it was reported that the essential AA composition of the empty body protein of female pigs (45 kg) was not affected by DE intake [(i.e. 2.5 versus 3.0 times DE for maintenance (DE_{maint})].

1.4.4. Protein retention and nitrogen and amino acid intake

Body weight gain of pregnant gilts and sows depends not only on energy but also AA intake (Gonçalves et al., 2016). The amounts of protein accretion that can be supported per gram of N, Lys, or Thr intake have been reported in several studies. Table 1-2 summarizes the relationship of these variables in pregnant pigs which suggests a linear-plateau response. Dourmad and Étienne (2002) obtained the maximum N retention at 10.5 and 6.3 g/d SID Lys and Thr, respectively. The value is lower than the findings of Srichana (2006) for Lys at 14.3 g/d for early and mid-gestation and 19.0 g/d for late gestation; but is higher than 5.0 g/d obtained by Leonard and Speer (1983) for Thr. King and Brown (1993) reported N retention was maximized at 36.3 g/d N intake. Similar

Table 1-2. Effect of Lys, Thr or N intake on N retention of pregnant pigs

AA or N Intake, g/d	N Retained, g/d	Animal, Method Used, Source
Lysine		
6.56	8.00	Pregnant Sows, N Balance, Dourmad and Étienne (2002)
8.55	11.90	
10.52	14.50	
12.47	14.70	
8.36	12.50	Pregnant Gilts (Early and Mid), N Balance, Srichana (2006)
11.22	19.13	
14.28	25.63	
17.40	26.83	
11.15	16.37	Pregnant Gilts (Late), N Balance, Srichana (2006)
14.96	26.50	
19.04	32.85	
22.85	31.40	
Threonine		
5.60	11.60	Pregnant Sows, N Balance Dourmad and Étienne (2002)
6.30	13.20	
7.00	13.40	
7.70	13.20	
3.59	5.20	Pregnant Gilts, N Balance, Leonard and Speer (1983)
4.95	8.10	
6.31	6.10	
7.67	7.90	
9.03	7.00	
Nitrogen		
11.20	4.00	Pregnant Gilts (Late), N Balance, King and Brown (1993)
17.20	6.50	
23.10	10.20	
29.00	12.50	
34.90	16.10	
40.80	16.00	
46.70	16.00	
52.60	18.00	

linear-plateau response has been demonstrated for lactating (King et al., 1993; Dourmad et al., 1998) and growing pigs (Patráš et al., 2012).

The N retention reported in the N balance studies (Table 1-2) represents the sum of protein gain in the maternal body and products of conception. Using a comparative slaughter technique, Everts and Dekker (1995) evaluated the effect of protein supply during pregnancy on the composition (i.e. water, protein and AA, lipid, and energy) of maternal body (includes mammary gland) and products of conception in gilts (piglets, placenta, uterus and intra-uterine fluids). Results showed that N intake (42 to 50 g/d versus 62 to 74 g/d) did not affect the composition of products of conception or the AA pattern of the protein content of unborn piglets (5.79 and 3.41 g Lys and Thr, respectively per 100 g CP). Gilts fed a diet with lower N deposited less maternal protein (52 versus 74 g/d) and more fat (206 versus 170 g/d) than the control group (Everts and Dekker, 1995). The earlier findings of Everts and Dekker (1995) support the conclusion of Miller et al. (2016) that the development of products of conception has a higher priority than maternal body during late gestation. When separated from maternal body, protein content of mammary parenchymal tissues were not affected by protein intake (Kusina et al., 1999).

In addition to dietary N and AA, the concept of ideal protein and balance of essential AA is crucial for the efficient utilization of dietary protein (Heger et al., 1999; Ji, 2004). This is particularly important for pregnant sows under restricted feed allowance and for lactating sows with limited feed intake. Kim et al. (2009) suggested that the ideal Lys:Thr:Val:Leu for sows during gestation are 100:79:65:88 and 100:71:66:95 for d 0 to 60 and d 60 to 114 of gestation, respectively. Sows fed a diet with an ideal AA pattern gained more weight (49.9 versus 39.2 kg) and lost less backfat (0 versus 1.40 mm) from d 30 to 109 of gestation than the control counterpart (Kim et al., 2009).

1.4.5. Estimation of protein retention using N-balance

Nitrogen is a main body component that is required for tissue protein synthesis and production of several nitrogenous compounds (i.e. hormones, immune mediators, neurotransmitters, etc.) involved in a variety of functions (Tessari, 2006). Therefore, body N should be both quantitatively and qualitatively normal to ensure normal body processes. Using this concept, N balance studies are performed to determine the biological values of different feed ingredients and the protein and AA requirements of the test species (Just et al., 1982). In swine studies, N balance is determined by feeding the pigs over a period of time and collecting feces and urine (Adeola, 2001). Adaptation period of 4 to 10 d is necessary to ensure that equilibrium has been achieved (Rand et al., 1976; Tessari, 2006; Levesque, 2010). The balance (or retention) is then calculated as the difference of N intake and N excretion from the feces and urine.

Tessari (2006) pointed out that N balance overestimates N intake and underestimates N losses. Specifically, these variations are attributed to the losses of N during collection and chemical analyses of feeds, feces and urine, waste of feeds, N gas loss after denitrification by the colonic microflora, and N losses through the skin and expired air (Just et al., 1982; Tessari, 2006). When compared to the slaughter technique, discrepancies of 14.7 to 16.7% were observed by Just et al. (1982). The difference was reduced by 50% when: (1) balloon catheters were used for urine collection, (2) N in feces and urine were analyzed using undried samples instead of heat-dried samples; and (3) acid was added to the urine and pH <2 was maintained. Conversely, losses in whole body analyses in slaughter technique may lead to underestimation of protein deposition (Just et al., 1982).

Nitrogen is converted to protein using the factor 6.25 based on the mean 16 g N content per 100 g protein published by Jones in the 1930's. Zein in corn and glycinin in soybean contain 16.1 and 17.5% N, respectively. Similarly, protein isolated from animal tissues contain 16% N (Jones, 1931). The N-to-protein conversion factor determined from total Kjeldahl N and obtained from pigs fed corn-soy diet are 6.41 and 5.54 (based on hydrated and anhydrous AA formula weight, respectively) (Dintzis et al., 1988). The factor 6.25 is used in NRC (2012).

1.5. Implication of understanding the model for estimating AA requirements and the factors that affect protein retention in pregnant pigs

The NRC (2012) is an improved model for estimating AA requirements of the pregnant pig. However, empirical studies on AA requirements of pregnant pigs are limited; thus assumptions based on studies in growing-finishing pigs are used in the model development. The assumption of the linear relationship of protein retention and ME intake has been confirmed in the earlier studies; but the constant response of protein retention to ME intake across days of gestation was not evident in these studies.

In contrast, the assumption of constant efficiency of utilizing SID AA throughout gestation needs to be evaluated. NRC (2012) estimated the efficiency of utilizing SID AA for protein retention at late gestation (d 90 to 114) and used the same value for early and mid-gestation. Nitrogen balance studies in pregnant sows revealed a linear increase in N retention with constant SID Lys and Thr intake and total tract N digestibility across d of gestation (Dourmad and Étienne, 2002; Miller et al., 2017). Results from these studies suggest that a constant efficiency value for AA utilization is not reflective of the changes in metabolic demand in pregnant pigs from early to late gestation. Similarly as in some commercial condition (i.e. when barns are bump feeding) energy intake as a multiple of maintenance changes from early to late gestation. Energy is known to influence N retention. The information on the dynamics of efficiency utilizing AA for protein retention is necessary to model accurate requirements for all essential AA. Standardized ileal digestible Lys and Thr should be given initial emphasis as these two AA are the most limiting in a corn-soybean meal fed pigs.

Reliable models allow for accomplishing precision feeding in gestating pigs. For nutritionists, this is important for diet optimization and nutrient excretion (i.e. waste) management. High or low efficiency (of AA utilization for protein retention) results in lower or higher AA requirement, respectively. Errors in efficiency estimates result in unnecessary cost and excess nutrients when underestimated whereas overestimation results to suboptimal growth and reproductive performance. For pork producers, a reliable model is essential for increased production efficiency through reduced feed cost and improved breeding herd performance (i.e. prolonged sow longevity and better reproduction performance) (Moehn et al., 2012; Kim et al., 2013). Reproductive failure is the major reason for early culling particularly in young sows while poor performance becomes more of an issue for sow removal above parity 3 (Stalder et al., 2004). Boyd et al. (2000) summarized the impact of nutrition on reproduction and advised a phase feeding strategy during pregnancy to accommodate embryo viability during early gestation, growth and recovery of body reserves, and exponential fetal and mammary growth during late gestation.

1.6. Summary

Feed costs in a breeding herd account for 12% of the cost of producing a market hog. Improvement in breeding herd efficiency; therefore, can have a significant influence in reducing overall production cost. This can partly be achieved through precision feeding where pregnant pigs are provided with nutrients sufficient to meet requirements for maternal growth and gain of conceptus with minimal excess. Precision feeding relies on mathematical models to predict the change in requirements during periods of differing nutrient demands (i.e. parity, stage of gestation). The recent edition of NRC (2012) Swine Nutrient Requirements provides improved models for estimating nutrient requirements of pregnant pigs because the models are mechanistic, dynamic and deterministic in representing the biology of nutrient and energy utilization at the whole-animal level. By necessity, the models contain empirical elements to test the consistency of model-generated nutrient requirements with observations from empirical studies. However, the paucity of data, particularly for gestating pigs, results in assumptions in model development.

Whole body Lys and Thr requirements represent the sum of those required for maintenance functions and protein retention. An efficiency factor is also used to account for minimum and inevitable Lys and Thr catabolism and between animal variability. Maintenance requirements for Lys and Thr include those in basal endogenous intestinal losses and skin and hair losses; while requirements for protein retention are based on CP mass and Lys and Thr composition of six gestation protein pools. The efficiency of utilizing SID Lys and Thr for maintenance functions and proteins retention is estimated at

0.75 and 0.81 and 0.49 and 0.53, respectively and the estimate of efficiency is assumed to be constant across period of gestation.

The assumption of constant efficiency of utilizing SID Lys and Thr for protein retention; however, may not reflect the change in metabolic demand in pregnant pigs from early to late gestation as protein retention is affected by day of gestation and energy (as a multiple of maintenance) intake. Therefore, a study evaluating the efficiency of utilizing SID Lys and Thr for whole body protein retention in pregnant pigs during early, mid and late gestation is warranted.

CHAPTER 2

Research Rationale, Objectives and Operational Definition

2.1 Research Rationale

Precision feeding provides an opportunity for efficient and sustainable pork production. This relies on detailed knowledge of nutrient requirements for diet optimization. In diet formulation, meeting the requirement for SID AA (particularly Lys and Thr) is the second highest contributor to the total feed formulation cost following energy. In gestating pigs, AA requirement represents the sum of those required for maintenance functions and for protein retention. Standardized ileal digestible Lys and Thr requirements for protein retention are based on CP mass and Lys and Thr compositions of the maternal body protein and the four pregnancy-associated protein pools (i.e. fetal tissue, mammary/udder tissue, placental tissue and uterine tissue). Efficiency factors are also used to account for minimum plus inevitable Lys and Thr catabolism and between-animal variability. These efficiencies are equivalent to 0.49 and 0.53 for SID Lys and Thr, respectively and are assumed to be consistent across days of gestation. Assumption of constant efficiency however may not reflect the dynamics of metabolic demand in pregnant pigs throughout gestation.

To our knowledge, no studies have been conducted evaluating the changes in the efficiency of utilizing SID Lys and Thr for whole body protein retention (k_{SID}Lys and k_{SID}Thr) in pregnant pigs across period of gestation. In contrast to earlier research that estimated k_{SID}Lys and k_{SID}Thr using common dietary SID Lys or Thr throughout gestation, our research used dynamic dietary SID Lys and Thr that consider the metabolic changes in pregnant pigs summarized in NRC (2012). We hypothesized that the k_{SID}Lys and k_{SID}Thr were higher during late than early gestation and mid gestation was intermediate.

This research will contribute to refinement of the NRC requirement model for gestating pigs that is essential for diet optimization and nutrient excretion management. Also, this research will help improve the evaluation of diet economics during gestation which is a key factor for overall farm efficiency.

2.2 Research Objectives

The objective of the experiment was to evaluate the efficiency of utilizing SID Lys and Thr for whole body protein retention in pregnant gilts. Specifically, this research aimed to compare N, Lys and Thr retention, and k_{SID}Lys and k_{SID}Thr during early, mid and late gestation. To achieve these objectives, SID Lys and Thr requirements specific to three periods in gestation, and determined from NRC (2012) model, were used in the calculation of experimental diets. The pregnant gilt was used as she serves as the foundation of a successful sow herd.

2.3 Operational Definition

The k_{SID}Lys and k_{SID}Thr represent the ratio of Lys and Thr retained and the SID Lys and Thr intake, respectively. Thus, the inefficiency of use accounts for: (1) inevitable plus minimum Lys and Thr catabolism, (2) endogenous Lys and Thr losses and minimum turn-over, and (3) between animal variability.

CHAPTER 3

Efficiency of utilizing standardized ileal digestible Lys and Thr for whole body protein retention in pregnant gilts during early, mid and late gestation[1]

R.A.S. Navales*, J. Dunn[†], J.K. Htoo[‡], K. Touchette[§], R.C. Thaler* and C.L. Levesque*[2]

*Department of Animal Science, South Dakota State University, Brookings, SD, [†]ADM Animal Nutrition, [‡]Evonik Nutrition & Care GmbH, and [§]Ajinomoto North America, Inc.

3.1 Abstract

Two experiments were conducted to evaluate the efficiency of utilizing SID Lys and Thr for whole body protein retention (k_{SID}Lys and k_{SID}Thr) in pregnant gilts. In Exp. 1, 45 gilts (PIC 1050, 158.0 ± 8.0 kg at d 39.4 ± 1 of gestation) in two groups were used in a 3-period N-balance study. Gilts were assigned to one of 4 diets set to provide 60, 70, 80 and 90% of the model-predicted daily SID Lys requirement for protein retention (NRC, 2012) in each of early (d 41-52, 10.44 g/d), mid (d 68-79, 9.60 g/d) and late gestation (d 96-107, 16.04 g/d). Diets contained 3300 kcal ME/kg and 11.6% CP; given at a rate of 2.13 kg/d in early and mid-gestation and at 2.53 kg/d during late gestation. The 12 d balance period (7 d adaptation; 5 d urine and fecal collection) was based on total urine collection using urinary catheters and determination of fecal N-digestibility

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[1] Research supported by funds from Foundation for Food and Agriculture Research: New Innovator Award, ADM Animal Nutrition, Ajinomoto North America, Inc. and Evonik Nutrition & Care GmbH

[2] Corresponding author: crystal.levesque@sdstate.edu

using indigestible marker. The SID Lys required for whole body protein retention was estimated using the NRC (2012) model and the predicted Lys content of each gestation pool. Lysine efficiency at each diet Lys level was calculated as the ratio of daily Lys retention and daily SID Lys intake. Growth and farrowing performance were analyzed as randomized complete block with diet as the fixed effect and group as the blocking factor. The linear and quadratic response in whole body N and Lys retention and Lys efficiency for each balance period was determined. The k_{SID}Lys was determined from the slope generated by regressing whole body Lys retention versus SID Lys intake, with y-intercept set to 0. In Exp. 2, 45 gilts (PIC 1050, 165.7 ± 13.6 kg at d 39.1 ± 2 of gestation) were assigned to one of 4 diets set to provide 60, 70, 80 and 90% of the model-predicted daily SID Thr requirement for protein retention (NRC, 2012) in each of early (6.46 g/d), mid (6.05 g/d) and late gestation (9.75 g/d). Animal management, N-balance procedure, data collection and calculation, and statistical analyses were patterned from Expt. 1. In Expt. 1, measured SID Lys was higher than formulated where 90% of SID Lys was 11.98, 11.25, and 17.47 g/d in early, mid and late gestation, respectively. In Expt. 2, measured SID Thr was lower than formulated where 60% of SID Thr was 5.28, 5.08, and 7.43 g/d in early, mid and late gestation, respectively. In early and mid-gestation, whole body N retention, as well as, Lys and Thr retention, were not affected by the dietary SID Lys and Thr. In late gestation, there was a linear increase (P <0.001) in whole body N, Lys and Thr retention. The k_{SID}Lys and k_{SID}Thr in late gestation were determined to be 0.54. The lack of response in whole body protein retention in early and mid-gestation may in partly reflect excess Lys and Thr intake. Lysine and Thr efficiency calculated at the lowest diet Lys and Thr were 0.49 and 0.32 in early gestation and 0.61 and 0.52 in mid-gestation, respectively. Based on the available evidence, k_{SID}Lys and k_{SID}Thr do not appear to be constant throughout gestation.

Keywords: lysine efficiency, pregnant gilts, protein retention, threonine efficiency

3.2. Introduction

Constant efficiency of utilizing SID Lys and Thr intake for whole body protein retention (k_{SID}Lys and k_{SID}Thr) in pregnant gilts is assumed across gestation in the model for estimating SID Lys and Thr requirements (NRC, 2012). Nitrogen balance studies of Dourmad and Étienne (2002) and Miller et al. (2017); however, revealed an increasing N retention with constant SID Lys and Thr intake and total tract N digestibility from early to late gestation. Results from these studies suggest that pregnant pigs become more sensitive to AA intake as the pregnancy progressed and therefore, constant efficiency may not reflect the changes in metabolic demand during pregnancy.

The k_{SID}Lys or k_{SID}Thr have been reported in earlier studies of King and Brown (1993); Pettigrew and Yang (1997); Miller et al. (2016); but as these studies were not focused on evaluation of efficiency, Lys or Thr level based on single AA level was used which may have confounded the efficiency estimate. Experimental diet Lys or Thr level based on the requirement during late gestation may depress the efficiency estimate in early and mid-gestation when used in these periods because of the excess Lys or Thr intake. Study of de Lange et al. (2001) revealed that excess AA intake resulted in higher fractional inevitable AA in growing pigs, and thus a lower efficiency of AA utilization.

This experiment therefore aimed to evaluate k_{SID}Lys and k_{SID}Thr in pregnant gilts using graded SID Lys and Thr levels corresponding to levels below the predicted requirements in early, mid and late gestation.

3.3. Materials and Methods

The experiment protocols were approved by the South Dakota State University Animal Care and Use Committee (16-074A and 16-091A) and followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). The two experiments were conducted from November, 2016 to May, 2017.

3.3.1. Animals and management

The experiments were conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD where 45 gilts (PIC 1050; 158.0 ± 8.0 kg at 39.4 ± 1 d of gestation) in 2 groups were used in Exp. 1 and 45 gilts (PIC 1050, 165.7 ± 13.6 kg at 39.1 ± 2 d of gestation) in 2 groups were used in Expt. 2. Gilts were housed in gestation stalls (0.61 m x 1.98 m) from breeding to d 110 of gestation and were offered a common gestation diet (3300 kcal ME/kg, 0.54% SID Lys and 0.40% SID Thr), except during N-balance periods. Feed allocation per day (i.e. 2.27 kg/d) was based on a target body condition score of 3.

At 110 d of gestation, gilts were transferred to farrowing crates (1.83 m x 2.44 m) until weaning at d 21 of lactation. Gilts were offered a common lactation diet (3300 kcal ME/kg, 0.93% SID Lys and 0.61% SID Thr), according to feed curve recommendations. Lactation feed was administered by an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS, USA) allowing daily intake up to 20% above the set curve for *ad libitum* intake based on historical herd performance. Gestation and lactation diets were provided in meal form. Water was provided *ad libitum*.

The pigs and facilities were checked twice daily by trained research unit manager and assistant manager and by the assigned graduate research assistant during the N-balance periods.

3.3.2. Dietary treatments

When confirmed pregnant at 21 d of gestation, gilts in Exp.1 were randomly assigned to one of 4 experimental diets: **Lys-1**, **Lys-2**, **Lys-3** and **Lys-4**. Experimental diets were set to provide 60, 70, 80 and 90% of the model-predicted daily SID Lys requirements for protein retention (NRC, 2012) in each of early (d 41-52, 10.44 g/d), mid (d 68-79, 9.60 g/d) and late gestation (d 96-107, 16.04 g/d). Similarly, in Exp. 2 gilts were randomly assigned to one of 4 experimental diets: **Thr-1**, **Thr-2**, **Thr-3** and **Thr-4**. The corresponding model-predicted daily SID Thr requirements for protein retention were 6.46, 6.05 and 9.75 g SID Thr/d in early, mid and late gestation, respectively. The summary of the targeted SID Lys and Thr levels of the experimental diets are shown in Table 3-1. Diets were formulated to contain 3300 kcal ME/kg, 11.6 % CP, 0.86% total calcium and 0.43% available phosphorus. To ensure that other essential AA were not limiting the response, the dietary essential AA levels other than Lys (Expt. 1) followed the NRC (2012) recommendations for gestating pigs based on an ideal ratio to Lys, with 40-70% overage. For Expt. 2, SID Lys was set at 25% above the NRC (2012) recommendation on a g/d basis. Essential AA other than Thr followed the NRC (2012) recommendations based on an ideal ratio to Lys, with at least 20% overage. Experimental diets were given in two equal meals (i.e. 0630 and 1430 h) at a rate of 2.13 kg/d during early and mid-gestation and at 2.53 kg/d during late gestation to ensure energy was not limiting the response to test AA level in late gestation.

Within each balance period, the desired levels of test AA were prepared by blending the ‘low’ and ‘high’ SID Lys (Expt. 1) and SID Thr (Expt. 2) master diets. Ingredient composition and nutrient content of the four low and high master diets are presented in Table 3-2. Titanium dioxide was included at 0.20% as an indigestible marker to calculate total tract N digestibility.

3.3.3. Data collections, chemical analyses and calculations

General observations. In both experiments, body weight (BW) of the gilts were measured within 24-h of the start and end of each N-balance period for the determination of daily Lys or Thr requirements for maintenance and gestation weight gain. At farrowing, litter size at birth (born alive, still birth and mummified) was recorded and all live born and still born piglets were weighed within 24-h of farrowing for estimation of pregnancy-associated Pd. Daily feed disappearance was monitored for feed spillage and feed refusal. Sow illness, lameness, reproductive failure and mortality, and clinical signs of infection over the course of catheterization were noted.

Nitrogen Balance. In both experiments, three 12-d N-balance periods were conducted starting at d 41, 68 and 96 of gestation. Each period consisted of 7-d diet adaptation and 5-d urine and fecal collection. Nitrogen balance observations were based on total urine collection using urinary catheters and determination of fecal N-digestibility using indigestible marker. Urine was collected as described by Miller et al. (2016). Prior to each collection, urinary catheters (Figure 3-1, Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA, USA) were lubricated and inserted flaccidly through the urethra and the balloon was inflated with 30 mL saline solution to retain the catheter in the bladder. Catheters were connected to closed containers using polyvinyl tubing (Fisherbrand Clear PVC Tubing, 4.88 mm inner diameter; Fisher Scientific Co., Birmingham, AL, USA) and urine collected (Figure 3-2). Sulfuric acid was added to the containers to maintain pH <3. A representative subsample (1% of the successful daily collection) were obtained, pooled within each collection period and stored at 4 °C until further analysis. Urine collection for each balance period was considered successful when at least 3 d of collections were accomplished. Urinary catheters were removed at the end of each N-balance period. Fecal samples were obtained by rectal palpation and daily collections were pooled per gilt and period and stored at -20 °C until further analysis.

Nutrient Analyses. A subsample of feed from every bag of experimental diet in both experiments were collected, pooled and homogenized per period and block. Prior to analyses, aliquots from urine samples were placed in 120 mL specimen cups; approximately 200 g of each experimental diet and freeze-dried feces were ground using rotor mill (Centrifugal Mill ZM 200; Retsch GmbH, Haan, Germany) with 0.50 mm sieve. Urine, freeze-dried feces and experimental diets were analyzed for N content using combustion method (Rapid N III, Elementar Analysensysteme, GmbH, Hanau, Germany); crude protein was calculated as N x 6.25. Dry matter and titanium dioxide content in feces and feeds were quantified according to Short et al. (1996). Absorbance of standard and samples were read using Spectra MAX 190 plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 408 nm wavelength. Amino acid and proximate compositions of the low and high Lys and Thr master diets per batch of mixed diet were completed by a commercial laboratory (ESCL, University of Missouri, Columbia, MO).

Calculations. The Lys efficiency (Expt. 1) and Thr efficiency (Expt. 2) were calculated for individual gilts consistent with Mercado et al. (2006) as the ratio of whole body Lys and Thr retention (g/d) and SID Lys and Thr intake (g/d). Nitrogen retention (g/d) was calculated from daily feed allowance and analyzed dietary N content, minus daily N excretion in feces and urine. Fecal N excretion (g/d) was calculated from N intake and total tract N digestibility, with the latter estimated using the indicator method (NRC, 2012). Daily whole body protein retention (g/d) was estimated as daily N retention x 6.25. Using NRC (2012) gestating sow model (Eq. 8-56 to 8-60), Pd in each pregnancy-associated protein pool (fetus, mammary, uterus and placenta plus uterine fluids) was calculated based on actual litter size (including stillborn) and actual piglet birth weight. These were subtracted from whole body protein retention to arrive at maternal Pd. The Lys (Expt. 1) and Thr (Expt. 2) content in whole body protein retention was estimated using the CP mass and the predicted Lys and Thr content of each gestation pool. Per 100 g CP, the Lys and Thr contents of maternal, fetal, uterine, placental and mammary tissues are: 6.74 and 3.71, 4.99 and 2.79, 6.92 and 4.22, 6.39 and 4.22 and 6.55 and 5.24 g, respectively.

Daily SID Lys and Thr intake was calculated as the product of daily feed intake (kg/d), measured Lys and Thr level of diet (g/kg) and SID coefficients (%). Standardized ileal digestibility of AA in each of the low and high Lys and Thr master diets were determined in a separate trial using eight cannulated growing pigs according to Stein et al. (2007, Eq. 2, 3 and 7).

The k_{SID}Lys and k_{SID}Thr for each N-balance period was estimated from the slope generated by regressing whole body Lys and Thr retention (g/d) versus SID Lys and Thr intake (g/d), with y-intercept set to 0.

3.3.4. Statistical Analyses

Gilt reproductive performance data were analyzed as randomized complete block with diet as the fixed effect and group (i.e. block) as the random effect using the PROC MIXED procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC). Differences among treatments were separated using PDIFF option with adjusted Tukey's test. The linear and quadratic response in N retention variables and Lys and Thr efficiency were tested within each balance period using the PROC GLM procedure of SAS. Assumption of homogeneity of variances and normality of residuals were confirmed a priori using the PROC GLM and PROC UNIVARIATE procedures in SAS, respectively. Least square means were calculated using the lsmeans procedure in SAS. The k_{SID}Lys and k_{SID}Thr which were estimated from the slope generated by regressing whole body Lys and Thr retention (g/d) versus SID Lys and Thr intake (g/d), with y-intercept set to 0, were determined using the regression procedure in R (Version 3.4.1). For all analyses, a $P < 0.05$ was considered significant and $0.05 < P < 0.10$ was considered a tendency.

3.4. Results

3.4.1. Animals and Experimental Diets

Forty out of 45 gilts used in Expt. 1 completed the trial. Four gilts (3, Lys-2; 1, Lys-3) were found not pregnant after the last N-balance period and one of the gilts assigned to Lys-2 aborted at d 83 of gestation. During early, mid and late gestation, four

(3, Lys-2; 1, Lys-4), one (Lys-3) and one (Lys-3) gilts, respectively were either unsuccessfully catheterized or had incomplete collection (<3 d). One of the gilts in Lys-4 had ileitis in mid-gestation but recovered and was used in late gestation. Two gilts (1 each of Lys-2 and Lys-4) had low litter size (<5 total born piglets) and were excluded in the calculation of N retention variables, Lys efficiency and k_{SID}Lys in late gestation. In the course of the N-balance periods, all gilts consumed their daily feed allocation except for one of the gilts in Lys-2 that went off-fed on the last d of collection in early gestation. In Exp. 2, all gilts completed the trial. During early gestation, one of the gilts in Thr-1 went off-fed on d 3 of collection due to fever resulting in <3 d of successful collection and was excluded in the calculation of N retention variables. Otherwise, all gilts consumed their daily feed allocation during early gestation. Two gilts in each of mid (1 each of Thr-1 and Thr-4) and late gestation (1 each of Thr-1 and Thr-2) were unsuccessfully catheterized. All gilts consumed their daily feed allocation during mid and late gestation.

The analyzed SID Lys levels of the 2 batches of master diets in Expt. 1 were higher than formulated (i.e. 0.40 versus 0.36% and 0.69 versus 0.66% SID Lys for low and high SID Lys master diets, respectively, Table 3-2). The Lys-1, Lys-2, Lys-3 and Lys-4 diets provided 9.04, 10.02, 11.00 and 11.98 g SID Lys/d in early gestation, 8.58, 9.47, 10.36 and 11.25 g SID Lys/d in mid-gestation, and 12.88, 14.41, 15.94 and 17.47 g SID Lys/d in late gestation, respectively. These levels represent 8.7 ± 1.6 percentage units above the targeted levels of 60 to 90% of the model-predicted SID Lys requirements for protein retention. Crude protein content of the low and high SID Lys master diets were 11.50 and 11.94%, respectively and were 98% of the formulated levels. The ratios of other essential AA to SID Lys were 61.2 ± 28.2% above the NRC (2012) recommendation for ideal ratio. In Exp. 2, the analyzed dietary SID Thr levels of the master diets were lower than formulated (i.e. 0.24 versus 0.25% and 0.40 versus 0.43% SID Thr for low and high SID Thr master diet, respectively, Table 3-2). The Thr-1, Thr-2, Thr-3 and Thr-4 diets, provided 5.28, 5.86, 6.45 and 7.04 g SID Thr/d in early gestation, 5.08, 5.67, 6.26 and 6.65 g SID Thr/d in mid-gestation, and 7.43, 8.37, 9.30 and 10.23 g SID Thr/d in late gestation, respectively. These levels represent 12.0 ± 1.6 percentage units below the targeted levels of 60 to 90% of the model-predicted SID Thr requirements for protein retention. Crude protein content of the low and high SID Thr diets were 11.19 and 11.13%, respectively and were 95% of the formulated levels. The SID Lys levels of the master diets are 27.5 ± 12.8% above the requirements (g/d basis), whereas the ratios of other essential AA (other than Thr) to SID Lys were 12.2 ± 29.3% above the NRC (2012) recommendation for ideal ratio. On a g/d basis, the other essential AA were 43.0 ± 39.6% above NRC (2012) recommendation.

3.4.2. Growth and Farrowing Performance

In Exp. 1, BW in each N-balance period and overall ADG between d 41 and 108 ± 1 of gestation were not different among Lys levels (Table 3-3). There was an effect of SID Lys intake on total litter size (*P* = 0.038); however, no difference was detected among treatments when based on adjusted Tukey’s test. There was an effect of Lys intake on piglet born alive (*P* = 0.015) where born alive was greater (*P* = 0.039) in Lys-3 than Lys-4 with Lys-1 and Lys-2 litters intermediate. Number of stillborn and mummified, and piglet birth weight were not affected by Lys level. Similarly, in Exp. 2, BW in each N-balance period and overall ADG between d 41 and 108 ± 2 of gestation were not different among Thr levels (Table 3-3). Total litter size, number of piglets born alive, stillborn and mummified, and piglet birth weight were not different among gilts fed experimental diets.

3.4.3. Nitrogen Balance (Exp.1)

Whole body N retention variables, pregnancy- and maternal-associated Pd, and Lys efficiency in gestating gilts fed Lys limiting diets in early, mid and late gestation are summarized in Tables 3-4 to 3-6. Across all N-balance periods, there was a positive linear increase (P <0.001) in SID Lys intake. Nitrogen digestibility increased in a quadratic function (P = 0.04) with dietary SID Lys in early gestation and increased linearly (P <0.04) in mid and late gestation. Urinary N increased linearly (P = 0.043) in early, tended to increase linearly (P = 0.093) in mid, and decreased linearly (P = 0.002) in late gestation. During early and mid-gestation, whole body N retention was not affected by the dietary SID Lys. In late gestation, whole body N retention linearly increased (P <0.001) with dietary SID Lys. Similarly, whole body Lys retention was not affected by SID Lys intake in early and mid- gestation and linearly increased (P <0.001) in late gestation. The increasing SID Lys intake and non-significant difference in Lys retention resulted in decreasing Lys efficiency among gilts (linear, P <0.005) in early and mid-gestation. The Lys efficiency among gilts in late gestation also decreased with increasing SID Lys intake (linear, P <0.05). There were minimal effects of SID Lys intake on pregnancy- and maternal-associated Pd and maintenance Lys requirement, except for maternal-associated Pd that increased linearly (P <0.001) with SID Lys intake during late gestation. During early, mid and late gestation, Lys efficiency of individual gilts ranged from 0.31 to 0.49, 0.43 to 0.61, and 0.51 to 0.57, respectively. The k_{SID}Lys in late gestation based on regression analysis was 0.54 (Figure 3-3, P <0.001, R^2 = 0.73).

3.4.4. Nitrogen Balance (Exp. 2)

Whole body N retention variables, pregnancy- and maternal-associated Pd, and Thr efficiency in gestating gilts fed Thr limiting diets in early, mid and late gestation are summarized in Tables 3-7 to 3-9. Across all N-balance periods, there was a positive linear increase (P <0.001) in SID Thr intake. Nitrogen digestibility was not affected by dietary SID Thr in all N-balance periods. Urinary N was not affected by the experimental diet during early and mid-gestation; but decreased linearly (P <0.001) in late gestation. During early and mid-gestation, whole body N retention was not affected by the dietary SID Thr. In late gestation, whole body N retention linearly increased (P <0.001) with dietary SID Thr. Similarly, whole body Thr retention was not affected by SID Thr intake in early and mid- gestation and linearly increased (P <0.001) in late gestation. Similar to Exp. 1, the increasing SID Thr intake and non-significant difference in Thr retention resulted in decreasing Thr efficiency among gilts (linear, P <0.05) in early and mid-gestation. The Thr efficiency among gilts in late gestation also decreased with increasing SID Thr intake (linear, P <0.005 and quadratic, P = 0.087). There were minimal effects of SID Thr intake on pregnancy- and maternal-associated Pd and maintenance Thr requirement, except for maternal-associated Pd that increased linearly (P <0.001) with SID Thr intake during late gestation. During early, mid and late gestation, Thr efficiency of individual gilts ranged from 0.22 to 0.32, 0.41 to 0.52, and 0.51 to 0.59, respectively. The k_{SID}Thr in late gestation based on regression analysis was 0.54% (Figure 3-4, P <0.001, R^2 = 0.72).

3.5. Discussion

The current study aimed to evaluate the efficiency of utilizing SID Lys and Thr intake for whole body protein retention in pregnant gilts during early, mid, and late gestation. The Lys (Exp. 1) and Thr (Exp. 2) efficiency were calculated for individual gilts as the ratio of Lys and Thr retention and SID Lys and Thr intake, respectively. The k_{SID}Lys and k_{SID}Thr were estimated for each N-balance period based on the slope generated from regressing whole body Lys and Thr retention as a function of SID Lys and Thr intake. For our current approach, graded levels of SID Lys and Thr moderately below (i.e. 60 to 90%) the model-predicted requirements were used. Moehn et al. (2004) reported that in growing pigs, Lys catabolism, which is a determinant of efficiency, was independent of Lys intake at moderate restriction (i.e. 10 to 30% below requirement). Correspondingly, de Lange et al. (2001) reported a constant fractional inevitable Thr catabolism at similarly moderate restrictions of Thr intake; but a sparing effect was reported as a reduced rate of Thr catabolism at severe restrictions (<60%) and an increased rate of catabolism was observed at Thr intake above requirement (>100%). To account for potential error in using a single AA level to estimate AA efficiency throughout gestation; the SID Lys and Thr levels of the experimental diets within N-balance periods were calculated from a dynamic estimate of requirements specific to each N-balance period.

The four gilts that were found open in Exp. 1 were all from the first group (i.e. block) and unlikely related to experimental diets; but to inexperience of newly trained barn staff in pregnancy checking. The first group in Exp. 1 was also the first batch of gilts in the new research facility of the university. Missing observations in both experiments were either due to unsuccessful catheterization or incomplete collection associated with health concerns. Similarly, the off-feeding observed in one of the gilts in each experiment was not diet-related.

A separate study using cannulated growing pigs was conducted to determine the SID AA of the master diets. The SID Lys coefficients in the low and high Lys diets were determined to be 85.18 and 91.59%, respectively and similar with the expected coefficient of 82.21 and 89.09% based on NRC (2012). The determined SID Thr coefficients of the low and high Thr diets were 73.54 and 73.83%. The observed SID Thr of low Thr diet was somewhat similar to expected value based on NRC (2012) at 79.48% but the high Thr diet was >10 percentage units below the expected value of 86.06%. Therefore, the AA digestibility coefficients of standard corn-soybean meal diet generated from NRC (2012) were used in the two experiments. In Exp. 1, the analyzed AA contents (expect for Lys) of the master low and high diets were above the daily requirements of pregnant gilts and thus were unlikely to limit the response to SID Lys. In Exp. 2, the ratio of some essential AA (other than Thr) to Lys were below the ideal ratio (NRC, 2012) and thus may have impacted the response to SID Thr. Kim and Easter (2003) argued that ideal AA pattern increases the efficiency of protein synthesis. However, when expressed on g/d basis, all essential AA (except Thr) in Exp. 2 are above the NRC (2012) recommendation. The analyzed CP which were 95 to 98% of the formulated levels were enough to supply the N required for the synthesis of non-essential AA at 30.7, 28.8 and 45.0 g/d for early, mid- and late gestation, respectively (NRC, 2012). Moreover, based on the calculated dietary ME and daily feed allocation, the diets provided 7.03 and 8.35 Mcal ME/d during early/mid, and late gestation, respectively. The daily ME intakes represented 1.5 ± 0.1 and 1.6 ± 0.1 times ME_{maint} and were within the recommended 7.0 Mcal ME/d for gestating gilts at constant feed intake (USPCE, 2010).

Reproductive performance and N retention were within expected ranges. Maternal body weight gain and farrowing performance were generally not impacted by dietary treatments. The difference in total litter size and born alive in Expt. 1 were more likely an unfortunate effect of randomization than dietary treatment because diets were provided beginning at d 41 of gestation when number of viable fetuses were already established (Geisert and Schmitt, 2002). Further, there was no difference in stillborn, mummies, or piglet birthweight. Retained N in both experiments were lower than reported by Miller et al. (2016) and higher than reported by King and Brown (1993). However, when adjusting for differences in diet CP (i.e. Miller et al., 2016) and d of gestation (i.e. King and Brown, 1993) and when expressed as a percent of N absorbed, N retention is comparable among Miller et al. (2016), King and Brown (1993), and the current study.

During early and mid-gestation, whole body N retention, and as a result whole body Lys and Thr retention, was not affected by experimental diets. This is a deviation from the expected linear increase in response typical for dose-response relationship at nutrient intake below requirements (Moughan and Fuller, 2003). In Exp. 1, the experimental diets provided 9.6 ± 1.0 percentage units more than the targeted levels of 60 to 90% of the model-predicted SID Lys requirements for protein retention. In Exp. 2, the experimental diets provided 12.7 ± 1.4 percentage units less than the targeted levels of 60 to 90% of the model-predicted SID Thr requirements for protein retention. In both experiment, the test AA are below the model-predicted requirements, whereas the other essential AA (g/d) are above the requirements. The lack of response in early and mid-gestation means a regression slope (i.e. efficiency of use estimate) cannot be determined and insinuates that Lys (Expt. 1) and Thr (Expt. 2) were not limiting during this period. Similarly, the decreasing Lys and Thr efficiency in early and mid-gestation support the hypothesis that Lys and Thr were not limiting in the respective diets. In a dose-response relationship, intake above the test AA requirements results in no change in N-retention (Moughan and Fuller, 2003). Everts and Dekker (1995) also concluded that depressed AA efficiency indicates an AA supply above requirement for maximum protein deposition. In Exp. 1, an increase in urinary N was also observed with increasing Lys intake in both early and mid-gestation indicating increased catabolism of excess AA. Additionally, Kim et al. (2005) revealed that the Lys needed for tissue gain of pregnant gilts from d 0 to 70 of gestation was 5.19 g/d. The lowest SID Lys intake less requirement for maintenance in the present study was 7.76 and 6.33 g/d in early and mid-gestation, respectively. In the case of Exp. 2, while the lack of response in N retention may indicate Thr intake at or near requirement, the lack of change in urinary N excretion in both early and mid-gestation may suggest another factor was limiting. Levesque et al. (2011) reported a Thr requirement of 5 to 6 g/d in early gestation consistent with over-feeding in the present study (i.e. 5.28 to 7.04 g/d and 5.08 to 6.65 g/d SID Thr in early and mid-gestation, respectively). It is unlikely that SID Lys was limiting the response to Thr because the SID Lys levels of the master diets are 27.5 ± 12.8% above the requirements (g/d basis). The observed imbalance in the ratio of some essential AA to Lys in the present study may provide explanation to the observed response, as feeding imbalanced mixture of AA affect protein synthesis (Kim and Easter, 2003). However there is very limited data on the ideal AA ratio in early gestation.

In late gestation, linear increase in whole body N retention, and consequently Lys and Thr retention, was observed; and this indicates that SID Lys and Thr levels were below requirement. Urinary N in both experiment decreased with increasing Lys and Thr intake indicating greater whole body retention as was observed and providing additional evidence that the Lys and Thr were limiting. Additionally, Kim et al. (2005), using serial slaughter technique, recommended 15.26 and 10.86 g/d true ileal digestible Lys and Thr, respectively, to support tissue accretion and maintenance in pregnant gilts. Samuel et al. (2012) and Levesque et al. (2011), using indicator AA oxidation technique, reported total Lys and Thr requirements of 17.4 and 12.3 to 13.6 g/d in late gestation in multiparous sows and first litter sows have higher AA requirements (NRC, 2012).

During late gestation, the k_{SID}Lys and k_{SID}Thr were the same for both AA at 0.54 and slightly higher than the estimate of NRC (2012) at 0.49 for Lys and 0.53 for Thr. When corrected for efficiency above maintenance, the values in the present study were 0.62 and 0.75 for Lys and Thr, respectively. Our results agrees reasonably with the corresponding values obtained by Everts and Dekker (1995) using slaughter technique at 0.59 and 0.67. While the Lys efficiency decreased with increasing Lys intake in this period, the difference between the lowest and highest efficiency is only 6 percentage units compared to 18 percentage units in early and mid-gestation. Our results agrees with the conclusion of Moehn et al. (2004) that at moderate Lys intake restriction, fractional inevitable Lys catabolism, which is a determinant of AA efficiency, is constant. In contrast, Thr efficiency decreased with increasing Thr intake suggesting the lower level of Thr may be approaching a severe restriction (de Lange et al., 2001). Based on analyzed Thr levels, the actual Thr intake in late gestation in the current study was approximately

10.7 ± 0.5% below the targeted levels and thus Thr intake at the lowest level may have affected the efficiency estimate. When the lowest level is removed, the linear effect of the experimental diets is no longer significant and the k_{SID}Thr is reduced to 0.53.

While a regression equation to estimate marginal Lys or Thr efficiency in early and mid-gestation is not possible, our present study provides evidence that the efficiency of utilizing SID Lys and Thr for whole body protein retention is not constant across gestation period. The Lys and Thr efficiency at the lowest Lys and Thr intake may provide some indication of the k_{SID}Lys and k_{SID}Thr in early and mid-gestation considering that fractional inevitable AA catabolism is constant at moderate AA intake restriction (de Lange et al., 2001; Moehn et al., 2004). The k_{SID}Lys may be in the range of 0.49 and 0.61 in early and mid-gestation and k_{SID}Thr in the range of 0.32 and 0.52 in early and mid-gestation, respectively. In both cases efficiency appears to increase in mid-gestation and is not consistent between AA.

3.6. Conclusion

The k_{SID}Lys and k_{SID}Thr in late gestation appears to be 0.54. Although the k_{SID}Lys and k_{SID}Thr in early and mid-gestation cannot be determined; when Lys and Thr efficiency from the lowest SID Lys and Thr intake in each of early, mid and late gestation are compared, the assumption of consistent efficiency is not reflective of the changes in metabolic demand of pregnant pigs during pregnancy. While marginal efficiency of AA use is similar between Lys and Thr in late gestation, deviation between AA may exist in early and mid-gestation. In addition, the NRC (2012) SID Lys and Thr requirements during early and mid-gestation appear to be over-estimated whereas the estimates during late gestation appear to be reasonably accurate.

Table 3-1. Targeted SID Lys and Thr levels of the experimental diets (g/kg)[1]

	Early **(d 41 to 52)**	**Mid** **(d 68 to 79)**	**Late** **(d 96 to 107)**
		Experiment 1	
Feed Allocation, kg/d	2.13	2.13	2.53
SID Lys Requirement[2], g/d	12.17	11.34	18.08
SID Lys Levels, g/kg			
Lys-1 (60%)	3.75	3.52	4.61
Lys-2 (70%)	4.24	3.97	5.24
Lys-3 (80%)	4.73	4.42	5.88
Lys-4 (90%)	5.22	4.87	6.51
		Experiment 2	
Feed Allocation, kg/d	2.13	2.13	2.53
SID Thr Requirement[2], g/d	8.59	8.20	12.31
SID Thr Levels, g/kg			
Thr-1 (60%)	2.82	2.71	3.32
Thr-2 (70%)	3.12	3.00	3.71
Thr-3 (80%)	3.43	3.28	4.09
Thr-4 (90%)	3.73	3.56	4.48

[1] Requirement for protein retention was calculated as the difference of total SID Lys or Thr requirement and SID Lys or Thr requirement for maintenance function (34.8 and 44.5 mg/kg $BW^{0.75}$, respectively). Dietary SID Lys (Exp.1) and Thr (Exp. 2) of the diets were calculated based on the desired levels of test AA (g/d) and the corresponding feed allocation within N-balance periods.

[2] Calculated using NRC (2012) Swine Nutrient Requirements. Sow performance was set as follows: BW at breeding = 140 kg, parity = 1, gestation length = 114 d, anticipated litter size = 12.5, anticipated birth weight = 1.4 kg/pig, average sow weight gain = 570 g/d, and feed intake = 2.13 kg/d at d 1 - 90 of gestation and 2.53 kg/d at d 90 - 110 of gestation.

Table 3-2. Ingredient composition and nutrient content of the four low and high master diets[3]

Items	Exp.1 – Lys		Exp. 2 – Thr	
	Low	**High**	**Low**	**High**
Ingredients, %				
Corn	85.38	84.83	84.69	85.75
Soybean Meal, 46%	7.50	8.60	4.35	6.30
Soybean Oil	1.00	1.00	2.65	1.50
Glutamic Acid	2.00		3.15	0.48
L-Lysine HCl		0.35	0.48	0.72
DL-Methionine	0.01	0.30	0.20	0.35
L-Threonine	0.08	0.36		0.15
L-Tryptophan	0.03	0.13	0.10	0.14
L-Valine		0.26	0.18	0.33
L-Isoleucine		0.18	0.13	0.22
Titanium Dioxide	0.20	0.20	0.20	0.20
Others[4]	3.81	3.81	3.88	3.88
Formulated Nutrient Content				
ME, kcal/kg	3,300.00	3,300.00	3,300.00	3,300.00
NE, kcal/kg	2,550.00	2,550.00	2,550.00	2,550.00
Crude Protein, %	11.75	12.11	11.70	11.73
Total Lys, %	0.44	0.74	0.72	0.97
SID Lys, %	0.36	0.66	0.65	0.90
Total Thr, %	0.45	0.75	0.32	0.50
SID Thr, %	0.38	0.67	0.25	0.43
Ratio to SID Lys				
SID Met+Cys	0.97	1.00	0.78	0.76
SID Thr	1.06	1.02	0.38	0.48
SID Trp	0.31	0.32	0.25	0.23
SID Val	1.14	1.03	0.82	0.79
Total Ca, %	0.85	0.85	0.85	0.86
Avail. P, %	0.34	0.34	0.34	0.34
Analyzed Nutrient Content				
Crude Protein, %	11.50	11.94	11.19	11.13
Total Lys, %	0.49	0.78	0.78	0.92

SID Lys[5], %	0.40	0.69	0.71	0.85
Total Thr, %	0.47	0.70	0.30	0.47
SID Thr[5], %	0.39	0.63	0.24	0.40
Ratio to SID Lys				
SID Met+Cys	0.83	0.86	0.69	0.76
SID Thr	0.98	0.91	0.34	0.47
SID Trp	0.35	0.32	0.24	0.27
SID Val	1.05	0.99	0.75	0.84

[3] Average analyzed nutrient content of 2 batches of feeds for Exp.1 and 1 batch of feeds for Exp. 2

[4] Other [% inclusion, (Exp. 1 and Exp. 2)]: calcium carbonate: 1.31 and 1.30, MCP: 1.80 and 1.88, salt: 0.50, mineral premix: 0.15 and vitamin premix: 0.05. Mineral premix provided (mg per kg diet): Zinc: 165.00, Iron: 165.00, Manganese: 43.50, Copper: 16.50, Iodine: 0.36 and Selenium: 0.30. Vitamin premix provided (per kg diet): Vitamin A: 11,022.93 IU, Vitamin D_3: 11,022.93 IU, Vitamin E: 95 IU, Vitamin B_{12}: 0.04 mg, Menadione: 4.41 mg, Riboflavin: 9.92 mg, D-panthothenic acid: 33.07 mg, Niacin: 55.24 mg, Folic acid: 4.42 mg, Pyridoxine: 15.16 mg, Thiamine: 3.31 mg and Biotin: 0.40 mg.

[5] Calculated from analyzed total Lys and Thr multiplied by digestibility coefficient of a standard corn-soybean meal diet (NRC, 2012)

Table 3-3. Weight gain and farrowing performance of gestating gilts fed lysine (Lys) or threonine (Thr) limiting diets

Variables	**60%**	**70%**	**80%**	**90%**	**SEM**	***P*-value**
	Lysine, Exp. 1					
No. of Gilts	11	8	10	11		
Gestation Weight Gain, kg	45.98	44.00	47.62	41.95	0.98	0.198
Average Daily Gain, kg/d	0.69	0.66	0.71	0.63	0.01	0.203
Farrowing Performance						
Total Litter Size[6]	15.10	12.60	15.60	11.90	0.50	0.038
Born Alive	14.50^{wx}	11.60^{wx}	14.70^{w}	10.90^{x}	0.47	0.015
Stillborn	0.64	1.00	0.90	1.00	0.22	0.928
Mummified	0.64	0.50	0.20	0.73	0.14	0.612
Birth Weight[7], kg	1.28	1.47	1.23	1.37	0.03	0.130
	Threonine, Exp. 2					
No. of Gilts	11	11	11	12		
Gestation Weight Gain, kg	41.27	38.82	38.18	41.08	0.95	0.595
Average Daily Gain, kg/d	0.62	0.58	0.57	0.61	0.01	0.598
Farrowing Performance						
Total Litter Size[6]	14.50	13.90	13.00	13.50	0.42	0.688
Born Alive	13.60	13.10	12.30	12.40	0.43	0.711
Stillborn	0.60	0.82	0.73	1.08	0.21	0.874
Mummified	0.45	0.82	0.45	0.42	0.15	0.762
Birth Weight[7], kg	1.39	1.41	1.39	1.27	0.04	0.571

[6] Sum of piglets born alive and stillborn (total litter size)

[7] Calculated as the average of measured BW at birth for each born alive and stillborn piglet per litter.

Means within a row lacking a common superscript $^{w, x, y, z}$ differ (*P*-value <0.0.5)

Table 3-4. Nitrogen retention variables and the lysine (Lys) efficiency in gestating gilts fed Lys limiting diets at early gestation (d 48 to 52)

Variables	**Lys-1 60%**	**Lys-2 70%**	**Lys-3 80%**	**Lys-4 90%**	**SEM**	***P*-value**	
						Linear	Quadratic
No. of gilts	11	5	10	10			
Initial Body Weight, kg	157.55	161.80	158.30	157.22	1.33		
Final Body Weight, kg	164.35	167.96	166.34	163.44	1.40		
Feed Intake, kg/d	2.13	2.09	2.13	2.13	0.01		
Nitrogen Intake, g/d	38.65	36.94	39.12	38.32	0.11		
SID Lys Intake, g/d	9.49	10.10	11.13	11.95	0.03	<0.001	0.097
Nitrogen Digestibility, %	82.46	82.76	84.05	82.72	0.21	0.210	0.040
Urine Nitrogen, g/d	20.53	21.59	23.33	22.81	0.61	0.043	0.455
Nitrogen Retention, g/d	11.34	9.01	9.55	8.89	0.67	0.122	0.456
Pregnancy Associated Pd, g/d	10.19	9.64	10.18	9.07	0.16	0.063	0.436
Fetal, g/d	4.09	3.68	4.08	3.26	0.12	0.062	0.431
Placental, g/d	1.40	1.26	1.40	1.12	0.04	0.064	0.441
Uterine, g/d	2.61	2.61	2.61	2.61	0.00	1.000	1.000
Mammary, g/d	2.09	2.09	2.09	2.09	0.00	1.000	1.000
Maternal Pd, g/d	60.71	46.63	49.50	46.47	4.17	0.149	0.433
Whole-body Lys Retention, g/d	4.70	3.73	3.95	3.69	0.28	0.128	0.448
Maintenance Lys Reqt, g/d	1.73	1.70	1.73	1.73	0.00	0.649	0.164
Lys Efficiency, %	49.09	35.99	35.48	30.82	2.61	0.003	0.347

Table 3-5. Nitrogen retention variables and the lysine (Lys) efficiency in gestating gilts fed Lys limiting diets at mid-gestation (d 75 to 79)

Variables	**Lys-1 60%**	**Lys-2 70%**	**Lys-3 80%**	**Lys-4 90%**	**SEM**	***P*-value**	
						Linear	Quadratic
No. of gilts	11	8	9	10			
Initial Body Weight, kg	173.73	174.25	175.44	170.40	1.58		
Final Body Weight, kg	178.73	180.38	180.33	174.20	1.46		
Feed Intake, kg/d	2.13	2.13	2.13	2.13	0.00		
Nitrogen Intake, g/d	37.16	37.99	38.32	37.48	0.06		
SID Lys Intake, g/d	8.06	9.09	10.12	11.15	0.00	<0.001	
Nitrogen Digestibility, %	83.10	83.08	84.41	83.96	0.19	0.034	0.536
Urine Nitrogen, g/d	18.22	18.30	19.53	19.44	0.30	0.093	0.853
Nitrogen Retention, g/d	12.55	12.97	12.68	11.95	0.32	0.441	0.339
Pregnancy Associated Pd, g/d	30.91	29.24	31.11	27.30	0.58	0.105	0.432
Fetal, g/d	19.73	18.08	19.93	16.18	0.57	0.106	0.432
Placental, g/d	0.29	0.26	0.29	0.24	0.01	0.124	0.477
Uterine, g/d	2.82	2.82	2.82	2.82	0.00	1.000	1.000
Mammary, g/d	8.08	8.08	8.08	8.08	0.00	1.000	1.000
Maternal Pd, g/d	47.52	51.82	48.15	47.41	2.01	0.765	0.458
Whole-body Lys Retention, g/d	4.93	5.14	4.98	4.74	0.14	0.514	0.359
Maintenance Lys Reqt, g/d	1.73	1.75	1.75	1.75	0.00	0.007	0.003
Lys Efficiency, %	61.35	56.93	49.41	42.62	1.42	<0.001	0.598

Table 3-6. Nitrogen retention variables and the lysine (Lys) efficiency in gestating gilts fed Lys limiting diets at late gestation (d 103 to 107)

Variables	Lys-1 60%	Lys-2 70%	Lys-3 80%	Lys-4 90%	SEM	*P*-value Linear	*P*-value Quadratic
No. of gilts	11	7	9	10			
Initial Body Weight, kg	190.72	191.45	190.40	187.80	1.55		
Final Body Weight, kg	203.18	205.09	205.26	201.70	1.49		
Feed Intake, kg/d	2.53	2.53	2.53	2.53	0.00		
Nitrogen Intake, g/d	44.12	44.37	44.10	45.01	0.05		
SID Lys Intake, g/d	12.70	14.48	16.25	18.03	0.00	<0.001	
Nitrogen Digestibility, %	84.47	85.73	86.09	85.86	0.38	0.038	0.132
Urine Nitrogen, g/d	18.04	15.85	15.30	14.90	0.34	0.002	0.275
Nitrogen Retention, g/d	19.09	21.85	22.60	23.74	0.37	<0.001	0.291
Pregnancy Associated Pd, g/d	71.99	71.18	70.74	66.03	1.07	0.093	0.475
Fetal, g/d	51.66	50.85	50.40	45.69	1.07	0.093	0.476
Placental, g/d	0.02	0.02	0.02	0.02	0.00	0.393	0.563
Uterine, g/d	2.79	2.79	2.79	2.79	0.00	1.000	1.000
Mammary, g/d	17.52	17.52	17.52	17.52	0.00	1.000	1.000
Maternal Pd, g/d	47.23	65.16	70.51	82.36	2.47	<0.001	0.491
Whole-body Lys Retention, g/d	7.11	8.28	8.61	9.17	0.15	<0.001	0.328
Maintenance Lys Reqt, g/d	2.04	2.04	2.03	2.05	0.00	0.749	0.035
Lys Efficiency, %	55.96	57.20	52.97	50.87	1.03	0.037	0.406

Table 3-7. Nitrogen retention variables and the threonine (Thr) efficiency in gestating gilts fed Thr limiting diets at early gestation (d 48 to 52)

Variables	**Thr-1 60%**	**Thr-2 70%**	**Thr-3 80%**	**Thr-4 90%**	**SEM**	***P*-value**	
						Linear	Quadratic
No. of gilts	10	11	11	12			
Initial Body Weight, kg	167.48	165.54	166.08	164.83	2.07		
Final Body Weight, kg	173.31	171.10	170.82	170.50	2.05		
Feed Intake, kg/d	2.13	2.13	2.13	2.13	0.00		
Nitrogen Intake, g/d	36.44	36.20	36.15	36.38	0.05		
SID Thr Intake, g/d	5.28	5.86	6.45	7.04	0.00	<0.001	
Nitrogen Digestibility, %	85.78	84.89	83.87	84.95	0.27	0.113	0.048
Urine Nitrogen, g/d	24.03	23.22	23.02	24.33	0.37	0.807	0.127
Nitrogen Retention, g/d	7.23	7.51	7.31	6.58	0.38	0.480	0.468
Pregnancy Associated Pd, g/d	10.43	10.09	9.74	9.62	0.16	0.067	0.741
Fetal, g/d	4.27	4.02	3.75	3.66	0.12	0.067	0.738
Placental, g/d	1.46	1.37	1.28	1.25	0.04	0.067	0.732
Uterine, g/d	2.61	2.61	2.61	2.61	0.00	1.000	1.000
Mammary, g/d	2.09	2.09	2.09	2.09	0.00	1.000	1.000
Maternal Pd, g/d	34.74	36.83	35.96	31.49	2.33	0.569	0.446
Whole-body Thr Retention, g/d	1.69	1.76	1.71	1.54	0.09	0.492	0.463
Maintenance Thr Reqt, g/d	2.13	2.13	2.13	2.13	0.00	0.490	0.944
Thr Efficiency, %	31.89	29.93	26.56	21.90	1.38	0.005	0.596

Table 3-8. Nitrogen retention variables and the threonine (Thr) efficiency in gestating gilts fed Thr limiting diets at mid-gestation (d 75 to 79)

Variables	**Thr-1 60%**	**Thr-2 70%**	**Thr-3 80%**	**Thr-4 90%**	**SEM**	***P*-value**	
						Linear	Quadratic
No. of gilts	10	11	11	11			
Initial Body Weight, kg	185.50	181.07	177.98	182.16	2.09		
Final Body Weight, kg	188.60	185.28	183.10	186.46	2.02		
Feed Intake, kg/d	2.13	2.13	2.13	2.13	0.00		
Nitrogen Intake, g/d	35.86	35.71	37.12	35.75	0.08		
SID Thr Intake, g/d	5.08	5.67	6.26	6.65	0.00	<0.001	
Nitrogen Digestibility, %	85.19	84.88	84.22	85.30	0.23	0.901	0.157
Urine Nitrogen, g/d	19.02	18.78	18.67	18.78	0.37	0.797	0.811
Nitrogen Retention, g/d	11.53	11.54	12.62	11.74	0.38	0.628	0.558
Pregnancy Associated Pd, g/d	31.57	30.56	29.26	29.06	0.59	0.125	0.754
Fetal, g/d	20.39	19.39	18.10	17.91	0.58	0.126	0.734
Placental, g/d	0.29	0.28	0.26	0.26	0.01	0.128	0.743
Uterine, g/d	2.82	2.82	2.82	2.82	0.00	1.000	1.000
Mammary, g/d	8.08	8.08	8.08	8.08	0.00	1.000	1.000
Maternal Pd, g/d	40.46	41.55	49.55	44.24	2.35	0.367	0.500
Whole-body Thr Retention, g/d	2.62	2.64	2.90	2.69	0.09	0.555	0.538
Maintenance Thr Reqt, g/d	2.15	2.16	2.15	2.16	0.00	0.395	0.703
Thr Efficiency, %	51.67	46.54	46.29	40.50	1.46	0.013	0.918

Table 3-9. Nitrogen retention variables and the threonine (Thr) efficiency in gestating gilts fed Thr limiting diets at late gestation (d 103 to 107)

Variables	**Thr-1 60%**	**Thr-2 70%**	**Thr-3 80%**	**Thr-4 90%**	**SEM**	***P*-value**	
						Linear	Quadratic
No. of gilts	10	10	11	12			
Initial Body Weight, kg	197.50	195.50	192.03	194.17	2.22		
Final Body Weight, kg	209.20	205.70	204.25	205.92	2.11		
Feed Intake, kg/d	2.53	2.53	2.53	2.53	0.00		
Nitrogen Intake, g/d	43.78	43.11	43.43	43.98	0.08		
SID Thr Intake, g/d	7.43	8.37	9.30	10.23	0.00	<0.001	
Nitrogen Digestibility, %	85.63	84.47	85.42	84.74	0.23	0.298	0.524
Urine Nitrogen, g/d	17.82	17.18	14.44	13.74	0.28	<0.001	0.948
Nitrogen Retention, g/d	19.68	19.24	22.65	23.53	0.25	<0.001	0.210
Pregnancy Associated Pd, g/d	73.78	72.01	67.79	66.69	1.52	0.081	0.925
Fetal, g/d	53.44	51.68	47.46	46.36	1.52	0.081	0.925
Placental, g/d	0.03	0.03	0.02	0.02	0.00	0.058	0.984
Uterine, g/d	2.79	2.79	2.79	2.79	0.00	1.000	1.000
Mammary, g/d	17.52	17.52	17.52	17.52	0.00	1.000	1.000
Maternal Pd, g/d	49.24	48.25	73.76	80.35	1.79	<0.001	0.315
Whole-body Thr Retention, g/d	4.36	4.27	5.10	5.31	0.05	<0.001	0.192
Maintenance Thr Reqt, g/d	2.57	2.56	2.54	2.55	0.00	<0.001	0.006
Thr Efficiency, %	58.59	51.02	54.81	51.90	0.63	0.009	0.087

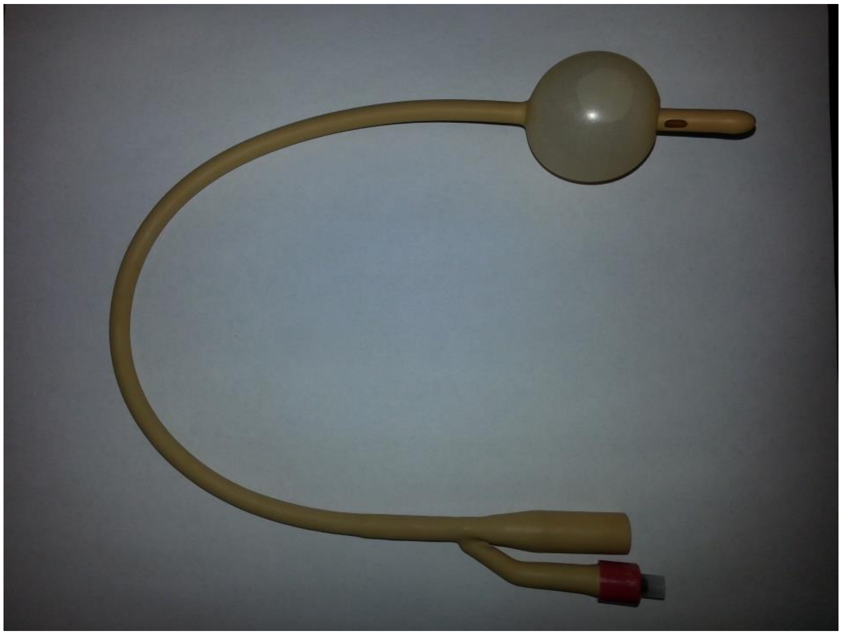

Figure 3-1. Urinary catheter used in the N-balance (Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA, USA)

Figure 3-2. Urine collection set-up. Urinary catheter was connected to closed container using polyvinyl tubing (Fisherbrand Clear PVC Tubing, 4.88 mm inner diameter; Fisher Scientific Co., Birmingham, AL, USA). Elastic band was used to suspend the tubing connection line off the floor and to alleviate any pressure off the bladder.

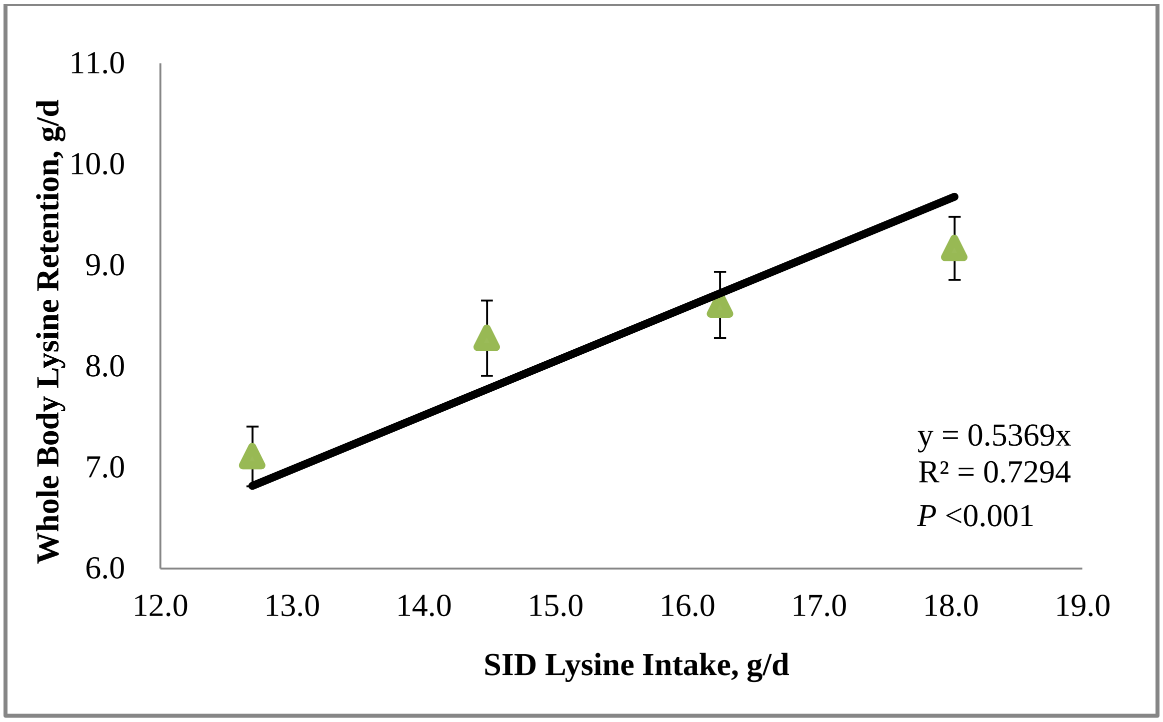

Figure 3-3. The efficiency of utilizing SID Lys intake for whole body Lys retention (k_{SID}Lys) at late gestation, estimated from the slope generated by regressing whole body Lys retention (g/d) versus SID Lys intake, with y-intercept set to zero.

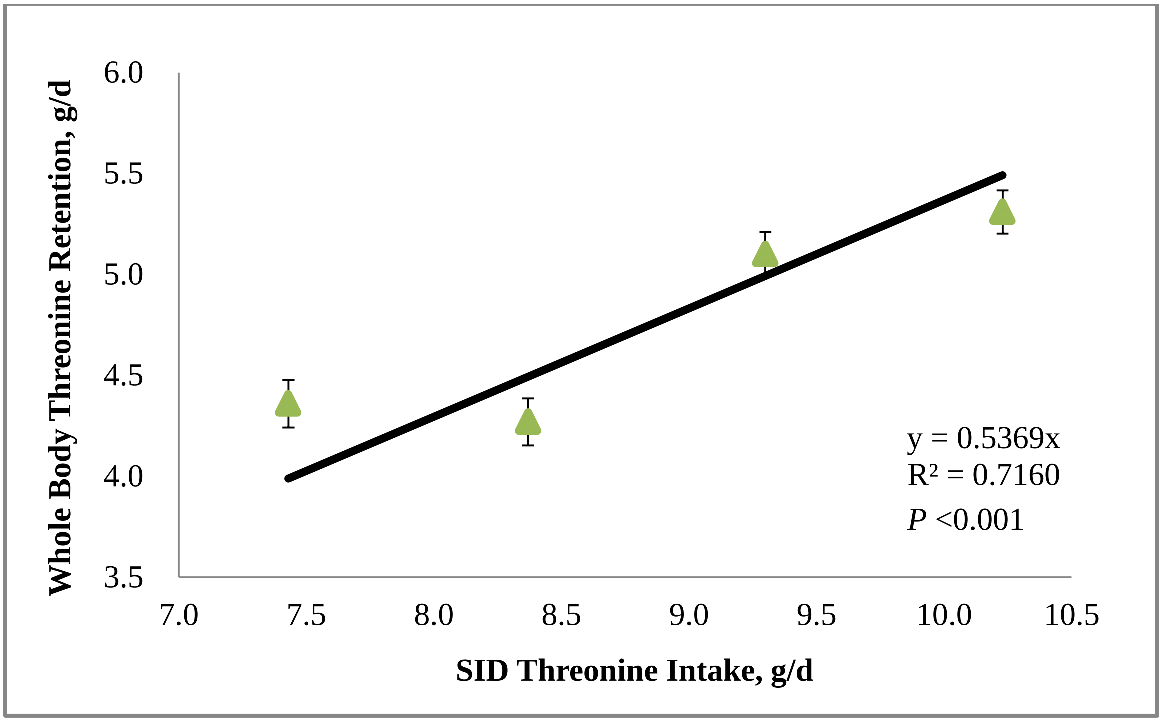

Figure 3-4. The efficiency of utilizing SID Thr intake for whole body Thr retention (k_{SID}Thr) at late gestation, estimated from the slope generated by regressing whole body Thr retention (g/d) versus SID Thr intake, with y-intercept set to zero.

CHAPTER 4

General Discussion

Precision feeding offers opportunity for improving swine herd efficiency and reducing overall production cost. In the breeding herd, precision feeding provides gestating pigs with nutrients sufficient to meet requirements for maternal growth and gain of conceptus with minimal excess and relies on mathematical models to estimate nutrient requirements at different stages of gestation. In Chapter 1, the main determinants of AA requirements of gestating pigs were defined and include requirements for basal endogenous gastrointestinal tract losses, integument losses, protein gain, and the efficiency of utilizing dietary nutrients for the aforementioned functions. Adjustments, particularly on the estimate of efficiency, have been made to match the model predicted with empirical requirements. The paucity of empirical studies in gestating pigs; however, resulted in the use of assumptions for model development (NRC, 2012). For AA, the model assumes that the efficiency of utilizing SID AA for protein retention is constant across period of gestation; but this is not reflective of the changes in metabolic demand in pregnant pigs from early to late gestation. Estimates of AA efficiency, particularly SID Lys and Thr, have been reported in earlier studies but single Lys and Thr levels were used in these studies.

Our current study (Chapter 3) aimed to evaluate the efficiency of utilizing SID Lys and Thr for whole body protein retention in pregnant gilts during early, mid and late gestation. The k_{SID}Lys and k_{SID}Thr during early and mid-gestation could not be determined because of the lack of response in Lys and Thr retention to increasing SID

Lys and Thr intake, respectively which reflects an oversupply of the respective test AA. During late gestation, the k_{SID}Lys and k_{SID}Thr were determined to be 0.54. Additionally, evidence from our present study suggest that the efficiency of Lys and Thr utilization for whole body protein retention is not constant throughout gestation when the lowest Lys or Thr intakes were compared among N-balance periods. From our current findings, we therefore conclude that the assumption of consistent efficiency is not reflective of the changes in metabolic demand of pregnant pigs during pregnancy.

The present study is not designed to evaluate SID Lys and Thr requirements of pregnant gilts; however, there is evidence from our research that requirements for SID Lys and Thr during early and mid-gestation are lower than the current NRC (2012) recommendation of 11 g SID Lys and 8 g SID Thr/d from d 0 to 90 of gestation. The requirement for SID Lys and Thr during late gestation (>90 d) appear to be reasonably represented in NRC (2012) at 17 and 12 g/d, respectively. In commercial production however, typical gestation diets and feeding levels provide approximately 10 to 12 g/d SID Lys (Goodband et al., 2013). Using the NRC (2012) ideal ratio to SID Lys this corresponds to 7 to 8 g SID Thr per d. Evidence from our current study suggest that in common industry practice, pregnant pigs are overfed with AA during early/mid gestation and are underfed during late gestation.

Follow-up studies to evaluate the dynamics of the efficiency of utilizing SID Lys and Thr intake for whole body protein retention throughout gestation at lower SID Lys and Thr levels (i.e. lower than 10 and 6 g/d) during early and mid-gestation in gilts and sows are warranted. Simultaneously, Ld can be used to validate the insufficiency of the test AA as restricted AA intake results to inflation of fat accretion. Moreover, evaluating the AA efficiency for protein retention in various gestation protein pools (i.e. gravid uterus and mammary tissues) is necessary for an accurate model development. Efficiency of AA utilization for protein retention during gestation should also be correlated to growth potential, especially for primaparous sows as they are still growing and maturing. For multiparous sows, body losses from previous lactation and the reconstitution of body reserve during subsequent pregnancy should be considered in the evaluation of N retention during pregnancy.

Our current research and the aforementioned research needs are key factors to the refinement of the AA requirement model for gestating pigs that are essential for diet optimization and nutrient excretion management. Errors in efficiency estimate, and hence the model, will result in unnecessary cost and excess nutrients when underestimated; whereas overestimation results to suboptimal growth and reproductive performance. Finally, refinement of the AA requirement model for gestating pigs will help swine producers in evaluating the diet economics of precision feeding to achieve total farm efficiency and sustainability.

LITERATURE CITED

Adeola, O. 2001. Digestion and Balance Techniques in Pigs. In: A. J. Lewis and L. L. Souther, editors, Swine Nutrition. CRC Press, Boca Raton, FL. p. 903-915.

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

Aherne, F. 2006. Feeding the Gestating Sow. http://porkgateway.org/resource/feeding-the-gestating-sow-2/ (Accessed January 14 2018).

Bazer, F. W., A. J. Clawson, O. W. Robison, C. K. Vincent, and L. C. Ulberg. 1968. Explanation for Embryo Death in Gilts Fed a High Energy Intake. Journal of Animal Science 27(4):1021-1026. doi: 10.2527/jas1968.2741021x

Bikker, P., M. Verstegen, and M. Bosch. 1994. Amino acid composition of growing pigs is affected by protein and energy intake. Journal of Nutrition 124:1961-1969.

Boyd, R. D., K. J. Touchette, G. C. Castro, M. E. Johnston, K. U. Lee, and I. K. Han. 2000. Recent Advances in Amino Acid and Energy Nutrition of Prolific Sows - Review. Asian-Australasian journal of animal sciences 13(11):1638-1652. doi: 10.5713/ajas.2000.1638

Calud, A. T., and K. Q. Tamisin. 2014. The 2013 swine production performance in the Philippines. In: I. Philippine Swine Industry Research and Development Foundation (ed.). p 20-25, Quezon City, Philippines.

Campbell, R. G., M. R. Taverner, and D. M. Curic. 1985. The effects of sex and of energy intake on rate of protein deposition in growing pigs. Proc. Aust. Soc. Anim. Prod. 15:660.

Chu, L. C., G. J. Zhang, C. J. Cai, X. B. Mao, and S. Y. Qiao. 2012. Effect of Dietary Energy Density on Performance and Lean Deposition of Growing-Finishing Pigs. Journal of Animal and Veterinary Advances 11(5):665-675. doi: 10.3923/javaa.2012.665.675

de Lange, C. F. M. 2013. New NRC (2012) nutrient requirements of swine. Advances in Pork Production 25:17-28.

de Lange, C. F. M., A. M. Gillis, and G. J. Simpson. 2001. Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets1. Journal of Animal Science 79(12):3087-3095. doi: 10.2527/2001.79123087x

Dintzis, F. R., J. F. Cavins, E. Graf, and T. Stahly. 1988. Nitrogen-to-Protein Conversion Factors in Animal Feed and Fecal Samples. Journal of Animal Science 66(1):5-11. doi: 10.2527/jas1988.6615

Dourmad, J.-Y., M. Étienne, A. Valancogne, S. Dubois, J. van Milgen, and J. Noblet. 2008. InraPorc: A model and decision support tool for the nutrition of sows. Animal Feed Science and Technology 143(1-4):372-386. doi: 10.1016/j.anifeedsci.2007.05.019

Dourmad, J. Y., and M. Étienne. 2002. Dietary lysine and threonine requirements of the pregnant sow estimated by nitrogen balance. Journal of Animal Science 80(8):2144-2150. doi: 10.2527/2002.8082144x

Dourmad, J. Y., M. Etienne, and J. Noblet. 1996. Reconstitution of body reserves in multiparous sows during pregnancy: effect of energy intake during pregnancy and mobilization during the previous lactation. Journal of Animal Science 74(9):2211-2219. doi: 10.2527/1996.7492211x

Dourmad, J. Y., M. Etienne, A. Prunier, and J. Noblet. 1994. The effect of energy and protein intake of sows on their longevity: a review. Livestock Production Science 40(2):87-97. doi: 10.1016/0301-6226(94)90039-6

Dourmad, J. Y., J. Noblet, and M. Etienne. 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. Journal of Animal Science 76(2):542-550. doi: 10.2527/1998.762542x

Everts, H., and R. A. Dekker. 1995. Effect of protein supply during pregnancy on body composition of gilts and their products of conception. Livestock Production Science 43(1):27-36. doi: doi.org/10.1016/0301-6226(95)00004-5

Geisert, R. D., and R. A. M. Schmitt. 2002. Early embryonic survival in the pig: Can it be improved? Journal of Animal Science 80(E-suppl_1):E54-E65. doi: 10.2527/animalsci2002.0021881200800ES10009x

Gonçalves, M. A. D., K. M. Gourley, S. S. Dritz, M. D. Tokach, N. M. Bello, J. M. DeRouchey, J. C. Woodworth, and R. D. Goodband. 2016. Effects of amino acids and energy intake during late gestation of high-performing gilts and sows on litter and reproductive performance under commercial conditions. Journal of Animal Science 94(5):1993-2003. doi: 10.2527/jas.2015-0087

Goodband, R. D., M. D. Tokach, M. A. D. Goncalves, J. C. Woodworth, S. S. Dritz, and J. M. DeRouchey. 2013. Nutritional enhancement during pregnancy and its effects on reproduction in swine. Animal Frontiers 3(4):68-75. doi: 10.2527/af.2013-0036

Heger, J., S. Mengesha, and D. Vodehnal. 1999. Effect of essential:total nitrogen ratio on protein utilization in the growing pig. British Journal of Nutrition 80:537-544. doi: 10.1017/S0007114598001639

Hill, G. M., and D. C. Mahan. 2016. Essential and nonessential amino acid compositions of the total litter and individual fetal pig content and accretion rates during fetal development. Journal of Animal Science 94(12):5239-5247. doi: 10.2527/jas.2016-0747

ISU. 2018. Estimated livestock returns. http://www2.econ.iastate.edu/estimated-returns/ (Accessed May 1 2018).

Ji, F. 2004. Amino acid nutrition and ideal protein for reproductive sows. PhD Dissertation, Texas Tech University.

Ji, F., G. Wu, J. R. Blanton, and S. W. Kim. 2005. Changes in weight and composition in various tissues of pregnant gilts and their nutritional implications. Journal of Animal Science 83(2):366-375. doi: 10.2527/2005.832366x

Ji, Y., Z. Wu, Z. Dai, X. Wang, J. Li, B. Wang, and G. Wu. 2017. Fetal and neonatal programming of postnatal growth and feed efficiency in swine. Journal of Animal Science and Biotechnology 8(1):42-56. doi: 10.1186/s40104-017-0173-5

Jin, S. S., S. W. Jung, J. C. Jang, W. L. Chung, J. H. Jeong, and Y. Y. Kim. 2016. Effects of dietary energy levels on the physiological parameters and reproductive performance of gestating gilts. Asian-Australasian journal of animal sciences 29(7):1004-1012. doi: 10.5713/ajas.16.0269

Jones, D. B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. https://organicroots.nal.usda.gov/download/CAT87213109/PDF (Accessed February 11 2018).

Just, A., J. A. Fernández, and H. Jørgensen. 1982. Nitrogen balance studies and nitrogen retention. In: J. P. Laplace, T. Corring and A. Rerat, editors, Digestive Physiology in Pigs No. 12. Institut National de la Recherche Agronomique. p. 207-215.

Kim, S. W., and R. A. Easter. 2003. Amino acid utilization for reproduction in sows. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. CABI Publishing, Oxon, UK. p. 203-222.

Kim, S. W., W. L. Hurley, G. Wu, and F. Ji. 2009. Ideal amino acid balance for sows during gestation and lactation. Journal of Animal Science 87(Suppl 14):E123-E132. doi: 10.2527/jas.2008-1452

Kim, S. W., A. C. Weaver, Y. B. Shen, and Y. Zhao. 2013. Improving efficiency of sow productivity: nutrition and health. Journal of Animal Science and Biotechnology 4(1):26. doi: 10.1186/2049-1891-4-26

Kim, S. W., G. Wu, and D. H. Baker. 2005. Ideal protein and amino acid requirements by gestating and lactating sows. Pig News and Information 26:89N-99N.

King, R. H., and W. G. Brown. 1993. Interrelationships between dietary protein level, energy intake, and nitrogen retention in pregnant gilts. Journal of Animal Science 71(9):2450-2456. doi: 10.2527/1993.7192450x

King, R. H., M. S. Toner, H. Dove, C. S. Atwood, and W. G. Brown. 1993. The response of first-litter sows to dietary protein level during lactation. Journal of Animal Science 71(9):2457-2463. doi: 10.2527/1993.7192457x

Krehbiel, C. R., and J. C. Matthews. 2003. Absorption of amino acids and peptides. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. CABI Publishing, Oxon, UK. p. 41-70.

Kusina, J., J. E. Pettigrew, A. F. Sower, M. R. Hathaway, M. E. White, and B. A. Crooker. 1999. Effect of protein intake during gestation on mammary development of primiparous sows. Journal of Animal Science 77(4):925-930. doi: 10.2527/1999.774925x

Leonard, R. P., and V. C. Speer. 1983. Threonine requirement for reproduction in swine. Journal of Animal Science 56(6):1345-1353. doi: 10.2527/jas1983.5661345x

Levesque, C. L. 2010. Evaluation of the threonine requirement and the biovailability of threonine in feedstuffs in pregnant sows. PhD Dissertation, University of Alberta.

Levesque, C. L., S. Moehn, P. B. Pencharz, and R. O. Ball. 2011. The threonine requirement of sows increases in late gestation. Journal of Animal Science 89(1):93-102. doi: 10.2527/jas.2010-2823

Lewis, C. R. G., and K. L. Bunter. 2013. A longitudinal study of weight and fatness in sows from selection to parity five, using random regression1. Journal of Animal Science 91(10):4598-4610. doi: 10.2527/jas.2012-6016

McPherson, R. L., F. Ji, G. Wu, J. R. Blanton, and S. W. Kim. 2004. Growth and compositional changes of fetal tissues in pigs. Journal of Animal Science 82(9):2534-2540. doi: 10.2527/2004.8292534x

Mercado, A., S. Leeson, S. Langer, B. Marty, and C. de Lange. 2006. Efficiency of utilizing ileal digestible lysine and threonine for whole body protein deposition in growing pigs is reduced when dietary casein is replaced by wheat shorts. Journal of Animal Science 84:1362-1374. doi: 10.2527/2006.8461362x

Miller, E. G., C. L. Levesque, N. Trottier, and C. F. M. de Lange. 2016. Dynamics of nitrogen retention in gestating gilts at two feeding levels. Journal of Animal Science 94(8):3353-3361. doi: 10.2527/jas.2016-0539

Miller, E. G., C. L. Levesque, N. L. Trottier, and C. F. M. de Lange. 2017. Dynamics of nitrogen retention at two feeding levels in gestating parity 2 and 3 sows1. Journal of Animal Science 95(6):2701-2710. doi: 10.2527/jas.2017.1444

Moehn, S., and R. O. Ball. 2013. Nutrition of Pregnant Sows. In: 13th London Swine Conference – Managing for Production, London, Ontario. p 55-63.

Moehn, S., R. O. Ball, M. F. Fuller, A. M. Gillis, and C. F. M. de Lange. 2004. Growth potential, but not body weight or moderate limitation of lysine intake, affects inevitable lysine catabolism in growing pigs. The Journal of nutrition 134(9):2287-2292. doi: 10.1093/jn/134.9.2287

Moehn, S., D. Franco, C. L. Levesque, R. Samuel, and R. O. Ball. 2012. Phase feeding for pregnant sows. Swine Research and Technology Centre, University of Alberta.

Moehn, S., D. Franco, P. B. Pencharz, and R. O. Ball. 2011. Dietary threonine requirement for maintenance is 98 mg/kg0.75 in adult sows. Advances in Pork Production Volume 22:Abstract #9.

Moughan, P. J. 1999. Protein metabolism in growing pig. In: I. Kyriazakis, editor, Quantitative Biology of the Pig. CABI Publishing, Wallingford, UK.

Moughan, P. J., and M. F. Fuller. 2003. Modelling amino acid metabolism and the estimation of amino acid requirements. In: J. P. F. D'Mello, editor, Amino acids in animal nutrition. CABI Publishing, Oxon, UK. p. 187-202.

Noblet, J., W. H. Close, R. P. Heavens, and D. Brown. 1985. Studies on the energy metabolism of the pregnant sow. British Journal of Nutrition 53(02):251-265. doi: 10.1079/bjn19850033

NRC. 1998. Nutrient requirements of swine: 10th revised edition. The National Academies Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine: 11th revised edition. The National Academies Press, Washington, DC.

Patráš, P., S. Nitrayová, M. Brestenský, and J. Heger. 2012. Effect of dietary fiber and crude protein content in feed on nitrogen retention in pigs. Journal of Animal Science 90(Suppl 4):158-160. doi: 10.2527/jas.53837

Pettigrew, J. E., and H. Yang. 1997. Protein nutrition of gestating sows. Journal of Animal Science 75(10):2723-2730. doi: 10.2527/1997.75102723x

Rand, W. M., V. R. Young, and N. S. Scrimshaw. 1976. Change of urinary nitrogen excretion in response to low-protein diets in adults. The American journal of clinical nutrition 29(6):639-644.

Samuel, R. S., S. Moehn, P. B. Pencharz, and R. O. Ball. 2008. Dietary lysine requirement for maintenance is 49 mg/kg0.75 in a population of modern, high productivity sows. Advances in Pork Production Volume 19:Abstract #1.

Samuel, R. S., S. Moehn, P. B. Pencharz, and R. O. Ball. 2012. Dietary lysine requirement of sows increases in late gestation. Journal of Animal Science 90(13):4896-4904. doi: 10.2527/jas.2011-4583

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology 59(4):215-221. doi: 10.1016/0377-8401(95)00916-7

Srichana, P. 2006. Amino acid nutrition in gestating and lactating sows. PhD Dissertation, University of Missouri, Columbia.

Stalder, K., M. Knauer, T. Baas, M. F. Rothschild, and J. Mabry. 2004. Sow longevity. Pig News and Information 25(2):53-74.

Stein, H. H., C. Pedersen, A. R. Wirt, and R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. Journal of Animal Science 83(10):2387-2395. doi: 10.2527/2005.83102387x

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. Journal of Animal Science 85(1):172-180. doi: 10.2527/jas.2005-742

Stein, H. H., N. L. Trottier, C. Bellaver, and R. A. Easter. 1999. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. Journal of Animal Science 77(5):1180-1187. doi: 10.2527/1999.7751180x

Tessari, P. 2006. Nitrogen Balance and Protein Requirements: Definition and Measurements. In: G. Mantovani, S. D. Anker, A. Inui, J. E. Morley, F. R. Fanelli, D. Scevola, M. W. Schuster and S.-S. Yeh, editors, Cachexia and Wasting: A Modern Approach. Springer Milan, Milano. p. 73-79.

USDA. 2018. Livestock and poultry: World markets and trade. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf. (Accessed May 01 2018).

USPCE. 2010. National swine nutrition guide: National swine nutrition guide tables on nutrient recommendations, ingredient composition, and use rates. US Pork Center of Excellence.

van Milgen, J., and J.-Y. Dourmad. 2015. Concept and application of ideal protein for pigs. Journal of Animal Science and Biotechnology 6(1):537-544. doi: 10.1186/s40104-015-0016-1

van Milgen, J., A. Valancogne, S. Dubois, J.-Y. Dourmad, B. Sève, and J. Noblet. 2008. InraPorc: A model and decision support tool for the nutrition of growing pigs. Animal Feed Science and Technology 143(1-4):387-405. doi: 10.1016/j.anifeedsci.2007.05.020

Whittemore, C. T., D. M. Green, and P. W. Knap. 2001. Technical review of the energy and protein requirements of growing pigs: protein. Animal Science 73(03):363-373. doi: 10.1017/s1357729800058331

Wu, G., T. L. Ott, D. A. Knabe, and F. W. Bazer. 1999. Amino acid composition of the fetal pig. Journal of Nutrition 129(5):1031-1038.

Xue, P. C., D. Ragland, and O. Adeola. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. Journal of Animal Science 92(9):3937-3944. doi: 10.2527/jas.2014-7815

Yen, J. T. 2001. Anatomy of the digestive system and nutritional physiology. In: A. J. Lewis and L. L. Souther, editors, Swine Nutrition. CRC Press, Boca Raton, FL. p. 31-63.

Zhai, H., and O. Adeola. 2011. Apparent and standardized ileal digestibilities of amino acids for pigs fed corn- and soybean meal-based diets at varying crude protein levels. Journal of Animal Science 89(11):3626-3633. doi: 10.2527/jas.2010-3732