Efficiency of utilizing standardized ileal digestible lysine and threonine for whole-body protein retention in pregnant gilts during early, mid-, and late gestation¹

Ronald A. S. Navales,* Jim Dunn,[†] John K. Htoo,[‡] Kevin Touchette,^{||} Robert C. Thaler,* and Crystal L. Levesque*,^{2,3}

*Department of Animal Science, South Dakota State University, Brookings, SD 57007; [†]ADM Animal Nutrition, Quincy, IL 62301; [‡]Evonik Nutrition & Care GmbH, Hanau 63457, Germany; and ^{||}Ajinomoto North America, Inc., Itasca, IL 60143

ABSTRACT: Two experiments were conducted to determine 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 (158.0 ± 8.0 kg at day 39.4 ± 1 of gestation) in 2 groups were used in a 3-period nitrogen (N)-balance study. Gilts were assigned to 1 of 4 diets set to provide 60, 70, 80, and 90% of predicted daily SID Lys requirement for protein retention (NRC, 2012) in each of early (day 41 to 52, 10.44 g/d), mid- (day 68 to 79, 9.60 g/d), and late gestation (day 96 to 107, 16.04 g/d). Diets contained 3,300 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 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 SID Lys intake. 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 vs. SID Lys intake, with y -intercept set to 0. In Exp. 2, 45 gilts (165.7 ± 13.6 kg at day 39.1 ± 2 of gestation) were assigned to 1 of 4 diets set to provide 60, 70, 80, and 90% of the predicted daily SID Thr requirement for protein retention 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 Exp. 1. In early and mid-gestation, whole-body N retention, as well as Lys and Thr retention, was 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 dietary Lys and Thr was 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.

Key words: lysine efficiency, pregnant gilts, protein retention, threonine efficiency

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²Corresponding author: crystal.levesque@sdstate.edu

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INTRODUCTION

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 this relies on accurate mathematical models. Numerous studies have determined nutrient requirements of growing pigs but there are limited empirical studies for the breeding herd where feed cost constitutes about 12% of the cost of producing a market hog (Calud and Tamisin, 2014). 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 AA requirements which is the second highest contributor to formula cost following energy requirements.

In gestating pigs, AA requirement represents the sum of those required for maintenance functions and for protein retention (NRC, 2012). Standardized ileal digestible (SID) Lys and Thr requirements for protein retention are factorially derived on crude protein (CP) mass and Lys and Thr compositions in the gain of maternal body protein and 4 pregnancy-associated protein pools (i.e., fetal tissue, mammary/udder tissue, placental tissue, and uterine tissue). Efficiency factors are also used to correct for the inefficiency of AA utilization for protein synthesis across the 4 pools. These efficiencies are equivalent to 0.49 and 0.53 for SID Lys and Thr, respectively (NRC, 2012). Constant efficiency of utilizing SID Lys and Thr intake for whole-body protein retention ($k_{\text{SID}}\text{Lys}$ and $k_{\text{SID}}\text{Thr}$) in pregnant gilts is assumed across gestation in the model for estimating SID Lys and Thr requirements (NRC, 2012). However, results from the N-balance studies of Dourmad and Étienne (2002) and Miller et al. (2017) suggest that pregnant sows and gilts become more sensitive to AA intake as gestation progresses. These are evident with the increasing N retention with constant SID Lys and Thr intake and total tract N digestibility from early to late gestation in these studies. Therefore, constant efficiency may not reflect the changes in metabolic demand during pregnancy. Moreover, the $k_{\text{SID}}\text{Lys}$ or $k_{\text{SID}}\text{Thr}$ during early, mid-, and late gestation have been reported in earlier studies of King and Brown (1993), Pettigrew and Yang (1997), and Miller et al. (2016). However, as the earlier studies were not focused on determining k_{SID} of a given AA, a single dietary SID Lys or Thr was used across periods which may have confounded the efficiency

estimate. The present study aimed to evaluate $k_{\text{SID}}\text{Lys}$ and $k_{\text{SID}}\text{Thr}$, the first 2 limiting AA, in pregnant gilts using graded dietary SID Lys and Thr corresponding to levels below the predicted requirements in different periods. To our knowledge, no studies have been conducted evaluating the changes in $k_{\text{SID}}\text{Lys}$ and $k_{\text{SID}}\text{Thr}$ in pregnant sows and gilts across period of gestation. We hypothesized that the $k_{\text{SID}}\text{Lys}$ and $k_{\text{SID}}\text{Thr}$ would increase with progressing pregnancy.

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 2 experiments were conducted from November, 2016 to May, 2017.

Animals and General Management

The experiments were conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD where 45 gilts in 2 groups of 22 and 23 animals each (PIC 1050; 158.0 ± 8.0 kg at 39.4 ± 1 d of gestation) were used in Exp. 1. An additional 45 gilts in 2 groups of 21 and 24 animal each (PIC 1050, 165.7 ± 13.6 kg at 39.1 ± 2 d of gestation) were used in Exp. 2. Gilts were housed in gestation stalls ($0.61 \text{ m} \times 1.98 \text{ m}$) from breeding to day 110 of gestation and were offered a common gestation diet (3,300 kcal ME/kg, 0.54% SID Lys, and 0.40% SID Thr) based on a target body condition score of 3 that was accomplished through visual scoring (i.e., 2.27 kg/d), except during nitrogen (N)-balance periods.

At 110 d of gestation, gilts were transferred to farrowing crates ($1.83 \text{ m} \times 2.44 \text{ m}$) until weaning at day 21 of lactation. Gilts were offered a common lactation diet (3,300 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) 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 research unit manager and assistant manager and by the assigned graduate research assistant during the N-balance periods.

Dietary Treatments

When confirmed pregnant at 21 d of gestation, gilts in Exp. 1 were randomly assigned to 1 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 (day 41 to 52, 10.44 g/d), mid- (day 68 to 79, 9.60 g/d), and late gestation (day 96 to 107, 16.04 g/d). These periods were selected based on Miller et al. (2017) where whole-body protein deposition increased linearly from day 52 of gestation. Mammary protein gain increases during mid-gestation (i.e., around 60 d of gestation) and an exponential increase in fetal protein gain occurs in the last trimester of gestation (Ji et al., 2005, 2006) further support the likelihood that efficiency of AA use during these periods is not constant. Similarly, in Exp. 2 gilts were randomly assigned to 1 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. Diets were formulated to contain 3,300 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 (Exp. 1) followed the NRC (2012) recommendations for gestating pigs based on an ideal ratio to Lys, with 40% to 70% overage. For Exp. 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 2 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 meet NRC (2012) energy intake requirement and 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 Lys-1 and Lys-4 diets for Exp. 1 (low and high Lys, respectively) and the Thr-1 and Thr-2 for Exp. 2 (low and high Thr, respectively). Ingredient composition and nutrient content of the low and high master diets for each experiment are presented in Table 1. Titanium dioxide was included at 0.20% as an indigestible marker to calculate total tract N digestibility.

Table 1. Ingredient composition and nutrient content of the 4 low and high master diets¹

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 ²	3.81	3.81	3.88	3.88
Formulated nutrient content				
ME, kcal/kg	3,300	3,300	3,300	3,300
NE, kcal/kg	2,550	2,550	2,550	2,550
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				
<i>SID Met+Cys</i>	0.97	1.00	0.78	0.76
<i>SID Thr</i>	1.06	1.02	0.38	0.48
<i>SID Trp</i>	0.31	0.32	0.25	0.23
<i>SID Val</i>	1.14	1.03	0.82	0.79
<i>SID Ile</i>	0.94	0.80	0.63	0.60
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 ³ , %	0.40	0.69	0.71	0.85
Total Thr, %	0.47	0.70	0.30	0.47
SID Thr ³ , %	0.39	0.63	0.24	0.40
Ratio to SID Lys				
<i>SID Met+Cys</i>	0.83	0.86	0.69	0.76
<i>SID Thr</i>	0.98	0.91	0.34	0.47
<i>SID Trp</i>	0.35	0.32	0.24	0.27
<i>SID Val</i>	1.05	0.99	0.75	0.84
<i>SID Ile</i>	0.93	0.80	0.55	0.64

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

²Other [% 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 D₃, 11,022.93 IU; vitamin E, 95 IU; vitamin B₁₂, 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.

³Calculated from analyzed total Lys and Thr multiplied to digestibility coefficient of a standard corn-soybean meal diet (NRC, 2012).

General Observations

In both experiments, 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 protein deposition (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 day 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 (Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA) 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 and urine collected. Sulfuric acid was added to the containers to maintain pH <3. A representative subsample (1% of the successful daily collection) was obtained, pooled within each 5-d collection period, and stored at 4 °C until further analysis. Urine collection for each balance period was considered successful when at least 3 complete 24-h 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 Analysis

A subsample of feed from every bag of experimental diet in both experiments was 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 $N \times 6.25$. 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) 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

Lysine (Exp. 1) and Thr (Exp. 2) efficiency at a given Lys or Thr intake was calculated for individual gilts, consistent with Libao-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. The N content of each experimental diet in each N-balance period was analyzed separately and used for the respective daily N intake. Fecal N excretion (g/d) was calculated from N intake and total tract N digestibility, with the latter estimated using the indicator method (Adeola, 2001). Daily whole-body protein retention (g/d) was estimated as daily N retention $\times 6.25$. Using NRC (2012) gestating sow model (Eqs. 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 (Exp. 1) and Thr (Exp. 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, Lys and Thr contents of maternal, fetal, uterine, placental, and mammary tissues were: 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 (NRC, 2012). 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 (%).

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

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 polynomial contrasts and the PROC GLM procedure of SAS. Assumption of homogeneity of variances and normality of residuals for gilt reproductive performance, N-retention variables, and Lys and Thr efficiency were confirmed a priori by Levene's and Shapiro-Wilk tests and using the PROC GLM and PROC UNIVARIATE procedures in SAS, respectively. Least square means were calculated using the lsmeans procedure in SAS. The $k_{\text{SID}}\text{Lys}$ and $k_{\text{SID}}\text{Thr}$ were determined from the slope generated by regressing whole-body Lys and Thr retention (g/d) against SID Lys and Thr intake (g/d), with y -intercept set to 0, using the regression procedure in R (Version 3.4.1). Litter size was included in the regression model and was determined to be not significant so was removed. For all analyses, a $P < 0.05$ was considered significant and $0.05 < P < 0.10$ was considered a tendency.

RESULTS

Animals and Experimental Diets

Forty out of 45 gilts used in Exp. 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 day 83 of gestation. A total of 6 gilts were either unsuccessfully catheterized or had incomplete collection (<3 d). They represented 4 gilts in early (3, Lys-2; 1, Lys-4), 1 in mid- (Lys-3), and 1 in late (Lys-3) gestation. One gilt in Lys-4 had ileitis 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 $k_{\text{SID}}\text{Lys}$ in late gestation. In the course of 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 day of collection in early gestation. In Exp. 2, all gilts completed the trial. During early gestation, 1 gilt in the Thr-1 group went off-fed on day 3 of collection due to fever resulting in <3 d of successful collection and was excluded in the calculation of N retention variables in that period. 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 Lys levels of the 2 batches of master diets in Exp. 1 were higher than formulated [i.e., 0.40 vs. 0.36% and 0.69 vs. 0.66% SID Lys for low and high SID Lys master diets, respectively (Table 1)]. 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 contents 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 at least 30% in excess of NRC (2012) requirements. In Exp. 2, the analyzed dietary Thr levels of the master diets were lower than formulated (i.e., 0.24 vs. 0.25% and 0.40 vs. 0.43% SID Thr for low and high SID Thr master diet, respectively, Table 1). 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 contents of the low and high SID Thr master diets were 11.19 and 11.13%, respectively, and were 95% of the formulated levels. The SID Lys levels of the master diets were $27.5 \pm 12.8\%$ above the requirements (g/d basis), whereas the ratios of other essential AA (other than Thr) to SID Lys were in excess of NRC (2012) requirements.

Growth and Farrowing Performance

In Exp. 1, BW in each N-balance period and overall ADG between day 41 and 108 ± 1 of

gestation were not different among Lys levels (Table 2). There was a main 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 day 41 and 108 ± 2 of gestation were not different among Thr levels (Table 2). Total litter size, number of piglets born alive, stillborn, and mummified, and piglet birth weight were not different among gilts fed experimental diets.

Nitrogen Balance (Exp. 1)

Whole-body N retention variables and individual Lys efficiency in early, mid-, and late gestation are summarized in Table 3. Nitrogen intake during mid- and late gestation increased linearly ($P < 0.001$), but not in early gestation. 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 nonsignificant difference in Lys retention resulted in decreasing Lys efficiency among treatments (linear, $P < 0.005$) in early and mid-gestation. The Lys efficiency among treatments 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,

Table 2. Weight gain and farrowing performance of gestating gilts fed lysine (Lys) or threonine (Thr) limiting diets

Variables ¹	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 ²	15.10 ^a	12.60 ^a	15.60 ^a	11.90 ^a	0.50	0.038
Born alive	14.50 ^{ab}	11.60 ^{ab}	14.70 ^a	10.90 ^b	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 ³ , 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 ²	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 ³ , kg	1.39	1.41	1.39	1.27	0.04	0.571

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

¹In each experiment, targeted test AA (i.e., Lys and Thr) intake was set at 60, 70, 80, and 90% of the respective requirement in early, mid-, and late gestation based on NRC (2012). The Lys-1, Lys-2, Lys-3, and Lys-4 diets provided 9.04, 10.02, 11.00, and 11.98 g/d in early gestation, 8.58, 9.47, 10.36, and 11.25 g/d in mid-gestation, and 12.88, 14.41, 15.94, and 17.47 g/d in late gestation, respectively. The Thr-1, Thr-2, Thr-3, and Thr-4 diets provided 5.28, 5.86, 6.45, and 7.04 g/d in early gestation, 5.08, 5.67, 6.26, and 6.65 g/d in mid-gestation, and 7.43, 8.37, 9.30, and 10.23 g/d in late gestation, respectively.

²Sum of piglets born alive and stillborn (total litter size).

³Calculated as the average of measured BW at birth for each born alive and stillborn piglet per litter.

Table 3. Nitrogen retention variables and the lysine (Lys) efficiency in gestating gilts fed Lys limiting diets at early (day 48 to 52), mid- (day 75 to 79), and late (day 103 to 107) gestation

Variables	Lys-1 60%	Lys-2 70%	Lys-3 80%	Lys-4 90%	SEM	P-value	
						Linear	Quadratic
Day 48 to 52							
No. of gilts	11	5	10	10			
Initial BW, kg	157.55	161.80	158.30	157.22	1.33		
Final BW, kg	164.35	167.96	166.34	163.44	1.40		
Nitrogen intake ¹ , g/d	38.65	36.94	39.12	38.32	0.11	0.269	
SID Lys intake ¹ , g/d	9.49	10.10	11.13	11.95	0.03	<0.001	
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
Whole-body Lys retention, g/d	4.70	3.73	3.95	3.69	0.28	0.128	0.448
Lys efficiency, %	49.09	35.99	35.48	30.82	2.61	0.003	0.347
Day 75 to 79							
No. of gilts	11	8	9	10			
Initial BW, kg	173.73	174.25	175.44	170.40	1.58		
Final BW, kg	178.73	180.38	180.33	174.20	1.46		
Nitrogen intake ¹ , g/d	37.16	37.99	38.32	37.48	0.06	<0.001	
SID Lys intake ¹ , g/d	8.06	9.09	10.12	11.15	—	<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
Whole-body Lys retention, g/d	4.93	5.14	4.98	4.74	0.14	0.514	0.359
Lys efficiency, %	61.35	56.93	49.41	42.62	1.42	<0.001	0.598
Day 103 to 107							
No. of gilts	11	7	9	10			
Initial BW, kg	190.72	191.45	190.40	187.80	1.55		
Final BW, kg	203.18	205.09	205.26	201.70	1.49		
Nitrogen intake ¹ , g/d	44.12	44.37	44.10	45.01	0.05	<0.001	
SID Lys intake ¹ , g/d	12.70	14.48	16.25	18.03	—	<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
Whole-body Lys retention, g/d	7.11	8.28	8.61	9.17	0.15	<0.001	0.328
Lys efficiency, %	55.96	57.20	52.97	50.87	1.03	0.037	0.406

¹Daily feed allocation was set at 2.13 kg/d in early and mid-gestation and 2.53 kg/d in late gestation.

except for maternal-associated Pd that increased linearly ($P < 0.001$) with SID Lys intake during late gestation (Supplementary Table 1). During early, mid-, and late gestation, Lys efficiency ranged from 0.31 to 0.49, 0.43 to 0.61, and 0.51 to 0.56, respectively. The k_{SID} Lys in late gestation based on regression analysis was 0.54 (Fig. 1, $P < 0.001$, $R^2 = 0.73$).

Nitrogen Balance (Exp. 2)

Whole-body N retention variables and individual Thr efficiency in early, mid-, and late gestation are summarized in Table 4. 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 and 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 nonsignificant difference in Thr retention resulted in decreasing Thr efficiency among treatments (linear, $P < 0.05$) in early and mid-gestation. The Thr efficiency among treatments in late gestation also decreased with increasing SID Thr intake (linear,

Figure 1. The marginal efficiency of utilizing standardized ileal digestible (SID) lysine (Lys) intake for whole-body Lys retention in pregnant gilts in late gestation (day 103 to 107) was estimated based on the slope generated from regressing whole-body Lys retention (g/d) as a function of SID Lys intake, with y -intercept set to 0. Graded levels of SID Lys moderately below (i.e., 60% to 90%) of the model-predicted requirement were used. The linear regression equation was: Whole-body Lys retention = 0.5369 (SID Lys intake); $R^2 = 0.729$, $P < 0.001$.

Table 4. Nitrogen retention variables and the threonine (Thr) efficiency in gestating gilts fed Thr limiting diets at early (day 48 to 52), mid- (day 75 to 79), and late (day 103 to 107) gestation

	Thr-1	Thr-2	Thr-3	Thr-4	SEM	P-value	
Variables	60%	70%	80%	90%		Linear	Quadratic
Day 48 to 52							
No. of gilts	10	11	11	12			
Initial BW, kg	167.48	165.54	166.08	164.83	2.07		
Final BW, kg	173.31	171.10	170.82	170.50	2.05		
Nitrogen intake ¹ , g/d	36.44	36.20	36.15	36.38	0.05	0.439	
SID Thr intake ¹ , g/d	5.28	5.86	6.45	7.04	—	<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
Whole-body Thr retention, g/d	1.69	1.76	1.71	1.54	0.09	0.492	0.463
Thr efficiency, %	31.89	29.93	26.56	21.90	1.38	0.005	0.596
Day 75 to 79							
No. of gilts	10	11	11	11			
Initial BW, kg	185.50	181.07	177.98	182.16	2.09		
Final BW, kg	188.60	185.28	183.10	186.46	2.02		
Nitrogen intake ¹ , g/d	35.86	35.71	37.12	35.75	0.08	0.167	
SID Thr intake ¹ , g/d	5.08	5.67	6.26	6.65	—	<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
Whole-body Thr retention, g/d	2.62	2.64	2.90	2.69	0.09	0.555	0.538
Thr Efficiency, %	51.67	46.54	46.29	40.50	1.46	0.013	0.918
Day 103 to 107							
No. of gilts	10	10	11	12			
Initial BW, kg	197.50	195.50	192.03	194.17	2.22		
Final BW, kg	209.20	205.70	204.25	205.92	2.11		
Nitrogen intake ¹ , g/d	43.78	43.11	43.43	43.98	0.08	0.159	
SID Thr intake ¹ , g/d	7.43	8.37	9.30	10.23	—	<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
Whole-body Thr retention, g/d	4.36	4.27	5.10	5.31	0.05	<0.001	0.192
Thr efficiency, %	58.59	51.02	54.81	51.90	0.63	0.009	0.087

¹Daily feed allocation was set at 2.13 kg/d in early and mid-gestation and 2.53 kg/d in late gestation.

$P < 0.005$ 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 (Supplementary Table 2). 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 (Fig. 2, $P < 0.001$, $R^2 = 0.72$).

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. Lysine (Exp. 1) and Thr (Exp. 2) efficiency was 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. In addition, 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.

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 Exp. 1 were more likely an effect of randomization than dietary treatment because diets were provided beginning at day 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 dietary CP (i.e., Miller et al., 2016) and day of gestation (i.e., King and Brown, 1993) and when expressed as a percent of N absorbed, N retention was comparable among Miller et al. (2016), King and Brown (1993), and the current study.

The observed linear response in N intake in mid- and late gestation in Exp. 1 which was not evident in early gestation was more likely a reflection of variation in diet sampling and laboratory analysis than difference between treatment levels because all diets were a blend of 2 master diets which had minor differences of 0.07 percentage units in analyzed N content.

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 retention typical for dose-response relationship at nutrient intake below requirements (Moughan and Fuller, 2003). In Exp. 1, 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, experimental diets provided

Figure 2. The marginal efficiency of utilizing standardized ileal digestible (SID) threonine (Thr) intake for whole-body Thr retention in pregnant gilts in late gestation (day 103 to 107) was estimated based on the slope generated from regressing whole-body Thr retention (g/d) as a function of SID Thr intake, with y -intercept set to 0. Graded levels of SID Thr moderately below (i.e., 60% to 90%) of the model-predicted requirement were used. The linear regression equation was: Whole-body Thr retention = 0.5369 (SID Thr intake); $R^2 = 0.716$, $P < 0.001$.

12.7 $\pm$ 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 experiments, 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 (Exp. 1) and Thr (Exp. 2) were not limiting during these periods. Similarly, the decreasing Lys and Thr efficiency on an individual gilt basis in early and mid-gestation supports 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. 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% of requirements) and an increased rate of catabolism was observed at Thr intake above requirement (>100%). Kim et al. (2005) revealed that intake of 5.19 g Lys/d is needed for tissue gain of pregnant gilts from day 0 to 70 of gestation. The lowest SID Lys intake for protein retention 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 overfeeding 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 diets are 27.5 $\pm$ 12.8% above NRC (2012) predicted requirement (g/d basis). Although there is very limited data on the ideal AA ratio in early gestation, the excess in AA:Lys ratio in master diets similarly suggests it unlikely another

essential AA may have been limiting the response to Thr in early and mid-gestation and further supports that protein deposition was maximized at the lower Lys and Thr intakes.

In late gestation, linear increase in whole-body N retention, and consequently Lys and Thr retention in combination with decreasing urinary N in both experiments with increasing Lys and Thr intake demonstrate that Lys and Thr were limiting in late gestation. 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, respectively, in multiparous sows.

During late gestation, the marginal efficiency of Lys and Thr based on regression analysis was the same for both AA at 0.54 and similar to the estimate of NRC (2012) at 0.49 for Lys and 0.53 for Thr. When corrected for efficiency above maintenance, the k_{SID} values in the present study were 0.62 and 0.75 for Lys and Thr, respectively. Our results agree reasonably with the corresponding values obtained by Everts and Dekker (1995) using slaughter technique at 0.59 and 0.67. While the k_{SID} Lys decreased with increasing Lys intake in this period, the difference between the lowest and highest efficiency is only 5 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, k_{SID} Thr 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 $\pm$ 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 marginal efficiency of Thr use is reduced to 0.53.

Additionally, the Lys and Thr efficiency of individual gilts at the lowest Lys and Thr intake in these periods may provide some indication of the marginal efficiency 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.

From our current findings, we therefore conclude that the assumption of consistent efficiency is not reflective of the changes in metabolic demand of pregnant sows and gilts during pregnancy. In addition, the [NRC \(2012\)](#) SID Lys and Thr requirements during early and mid-gestation appear to be overestimated, whereas the estimates during late gestation appear to be reasonably accurate. Our current research is a key factor 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 in suboptimal growth and reproductive performance. Finally, refinement of the AA requirement model for gestating pigs will help swine producers in evaluating the economics of precision feeding to achieve total farm efficiency and sustainability.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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