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The threonine requirement of sows increases in late gestation

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ABSTRACT: Current AA recommendations for sows are to provide a fixed amount of AA intake throughout gestation based on the assumption that there is a constant demand for AA; however, the demand for nutrients changes from maternal lean tissue in early gestation to fetal and mammary growth in late gestation. The objective of this study was to determine the Thr requirement in early (d 35 to 53 and 25 to 55 for Exp. 1 and 2, respectively) and late (d 92 to 110 and 81 to 111 for Exp. 1 and 2, respectively) gestation using the indicator AA oxidation (IAAO) method with L-[1-13C]Phe as the tracer AA. A total of 14 multiparous sows were used: 6 in Exp. 1 and 8 in Exp. 2. Each sow received each of 6 diets in random order in both early and late gestation. A basal diet was formulated to contain Thr at 60% of the 1998 NRC recommendation in Exp. 1 and 20 and 60% of the 1998 NRC in Exp. 2 for early and late gestation, respectively. Crystalline l-Thr was added to create additional diets with approximately 10% incremental increases in Thr. Sows were placed in respiration chambers, and expired air and blood were collected every 30 min for 5.5 h. Tracer Phe [mg/(kg of BW·h)] was given orally over the last 4 h divided into eight 0.5-h meals. Expired air and plasma were measured for 13CO2 enrichment and free Thr concentration, respectively. Background 13CO2 was subtracted from plateau 13CO2 enrichment. Data were analyzed using a 2-phase nonlinear Mixed model. The overall litter size and litter weight were 13.5 ± 3.1 and 20.5 ± 3.9 kg, respectively. Based on IAAO, the Thr requirement in early gestation was 6.1 g/d (R² = 0.59, Exp. 1) and 5.0 g/d (R² = 0.71, Exp. 2). In late gestation, the Thr requirement based on IAAO was 13.6 g/d (R² = 0.60, Exp. 1) and 12.3 g/d (R² = 0.58, Exp. 2). Based on plasma Thr, the Thr requirement in early gestation was 7.0 g/d (R² = 0.90, Exp. 1) and 3.9 g/d (R² = 0.90, Exp. 2). In late gestation, the Thr requirement based on plasma Thr was 10.5 g/d (R² = 0.67, Exp. 2). There was a linear response to increasing Thr intake in late gestation in Exp. 1. Feeding a single amount of AA throughout gestation results in overfeeding AA in early gestation and underfeeding AA in late gestation. The 2-fold increase in Thr requirement in the last third of gestation suggests that phase feeding sows in gestation will more closely meet the demands for nutrients and that the requirement for essential AA in gestating sows should be re-evaluated in early and late gestation separately.

Key words: gestation, requirement, sow, l-threonine

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INTRODUCTION

Recommendations for daily AA intake during gestation are based on maintenance and growth data from the 1970s and 1980s (NRC, 1998). Genetic selection has increased lean yield 1.5% and litter size by 30% from 1999 to 2006 (CCSI, 2007). These genetic improvements in lean yield and reproductive traits indicate that the AA requirements for sows in gestation are likely greater than current recommendation. Additionally, NRC (1998) recommends a fixed amount for AA requirements during gestation, which assumes a constant demand for AA throughout gestation. However, the metabolic focus of the sow changes from the recovery of body tissue after weaning to the synthesis of fetal tissue in late gestation (McPherson et al., 2004). Fetal weight, fetal protein content, and mammary protein content increase 5-, 18-, and 27-fold, respectively, in the last 45 d of gestation (McPherson et al., 2004; Ji et al., 2006). These dramatic increases in fetal weight and protein gain indicate that the requirement for AA

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may also be greater in late gestation compared with early gestation.

The daily demand for Thr may change to a greater extent from early to late gestation than the requirement for Lys. First, the growth of sows in pregnancy leads to an increase in maintenance requirements, which are greater for Thr than Lys (NRC, 1998). Second, the growth of sows in late gestation shift from maternal body protein gain to mucosal membranes (i.e., intestinal and mammary tissue) with increased concentrations of Thr in mucosal protein (Specian and Oliver, 1991). Along with the 27-fold increase in mammary tissue (Ji et al., 2006), the ratio of gastrointestinal tract to total fetal BW increases 1.6-fold in late gestation (McPherson et al., 2004). Therefore, Thr was most likely to reflect the principle of changing whole body AA requirements in gestation. The objective of this study was to determine the requirement for Thr in early and late gestation in sows.

**MATERIALS AND METHODS**

Animal care and experimental procedures were the same for each experiment and were approved by the University of Alberta Animal Policy and Welfare Committee.

The study was conducted to determine the response of indicator AA oxidation (IAAO) in early (EG; d 35 to 53 and 25 to 55 in Exp. 1 and 2, respectively) and late gestation (LG; d 92 to 110 and 81 to 111 in Exp. 1 and 2, respectively) to increasing amounts of dietary Thr, ranging from deficient to excess for gestating sows (approximately 10 g of total Thr/d; NRC, 1998). In Exp. 1, 6 Large White × Landrace gestating sows (second parity; 172 ± 16 kg of BW) were each fed 6 diets of increasing dietary Thr in a 6 × 6 Latin square design. The results of Exp. 1 indicated that the requirements for Thr in EG and LG were at the lower and upper end, respectively, of Thr intakes tested so that requirements could not be estimated with confidence. Therefore, the study was repeated (Exp. 2) using 6 sows and 6 diets in an unbalanced 8 × 6 Latin square design. Four sows from Exp. 1 (fourth parity) plus 4 additional sows (third parity) with a mean BW of 211 ± 21 kg were used.

**Experimental Diets**

The basal diets were based on corn, cornstarch, and sugar (Table 1). Corn was included to meet the least amount of dietary Thr within each basal diet. Thus, the limited amount of corn in the basal diet in EG in Exp. 2 led to greater inclusion of cornstarch and sugar because dietary Thr was set to meet only 20% of NRC (1998). Synthetic Thr was used to create diets with incremental increases (approximately 10%) in dietary Thr at the expense of cornstarch and sugar. All other essential AA were provided at a minimum of 150% (Exp. 1) and 180% (Exp. 2) of their respective NRC (1998) requirement. In Exp. 1, each sow received 3 amounts of dietary Thr below (60 to 80%) and 3 amounts above (110 to 130%) the NRC (1998) recommendation for second parity sows of similar BW, expected litter size, and maternal BW gain. In Exp. 2, sows received 3 amounts of dietary Thr above and 3 amounts below the Thr requirement estimated in Exp. 1, which was approximately 50 and 130% of NRC (1998) in EG and LG, respectively. Therefore, separate basal diets were formulated for EG and LG in Exp. 2 where dietary Thr was set at 20 and 60% of the NRC (1998) recommendation in EG and LG, respectively. The vitamin-mineral premix used in Exp. 1 was no longer available when the diets for Exp. 2 were formulated. The inclusion of NaCl, NaHCO3, KCl, and KHCO3 differed among diets to balance dietary electrolytes.

Dietary Phe concentration was kept constant in all diets. To facilitate channeling toward oxidation of any tyrosine synthesized from Phe (Shiman and Gray, 1998), dietary Tyr was set at 180% of the NRC (1998). The sows were provided their daily feed allowance in 2 equal daily feedings except on respiration days when one-half the daily allowance was divided into twelve 0.5-h portions based on Moehn et al. (2004a; Figure 1). Feed allowance was set to achieve the recommended daily energy intake based on BW and backfat at breeding according to Aherne and Foxcroft (2000). Sows were determined to be in a negative energy balance in LG in Exp. 1; thus, dietary energy content was increased in LG in Exp. 2 to achieve an increase in daily energy intake of approximately 6% (Samuel et al., 2007). Cellulose (Solka-Floc, International Fiber Corporation, Urbana, IL) and canola oil were altered to achieve the desired dietary energy content. All animals were individually housed and acclimated to metabolism pens and the first diet in their rotation for 7 d before the first respiration day. A minimum of 3 d of adaptation was used for each successive diet based on the reports that this was more than adequate adaptation period for respiration experiments (Moehn et al., 2004a; Elango et al., 2009). Experimental design and statistical analysis were the same for each experiment.

**Administration of Labeled AA**

The intracellular free Phe pool is tightly regulated (Flaim et al., 1982) and responds rapidly to changes in test AA intake (Neale and Waterlow, 1974); therefore, L-[1-13C]Phe was selected as the tracer AA. In Exp. 1, sows received an oral dose of 1 mg/(kg of BW·h) of L-[1-13C]Phe (99% enrichment, Sigma Aldrich, Mississauga, Ontario, Canada) for 4 h divided into 8 0.5-h feedings (Figure 1). A priming dose equal to 1.75 times the hourly dose was given along with the first 0.5-h dose. The sows consumed all the feed provided before administration of the next 0.5-h feed allowance. In Exp. 2, L-[1-13C]Phe hourly dose was increased to 2 mg/(kg of BW·h) to reduce variation in enrichment during isotopic plateau.
Data were collected from d 35 to 53 and from d 92 to 110 of gestation to represent EG and LG, respectively, in Exp. 1. In Exp. 2, data were collected from 25 to 55 d and from 81 to 111 d gestation to represent EG and LG, respectively. Before initiation of the study, sows were fitted with a surgically implanted coated catheter (CBAS 100 cm catheter, Instech Solomon, Plymouth Meeting, PA) and vascular access titanium injection port (TiSoloPort MAX, Instech Solomon; Swindle et al., 2005). Sows were allowed a minimum of 21-d recovery time before starting the study.

Apparent oxidation of Thr was measured in an aliquot of CO₂ from expired air after administration of L-[^13]C Phe. Two independent airtight respiration chambers (2.0 m²) were constructed in a temperature-controlled room, each using a standard farrowing crate with a rear

Sample Collection

Data were collected from d 35 to 53 and from d 92 to 110 of gestation to represent EG and LG, respectively, in Exp. 1. In Exp. 2, data were collected from 25 to 55 d and from 81 to 111 d gestation to represent EG and LG, respectively. Before initiation of the study, sows were fitted with a surgically implanted coated catheter (CBAS 100 cm catheter, Instech Solomon, Plymouth Meeting, PA) and vascular access titanium injection port (TiSoloPort MAX, Instech Solomon; Swindle et al., 2005). Sows were allowed a minimum of 21-d recovery time before starting the study.

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### Table 1. Composition and nutrient contents of basal diets fed to sows in early (d 25 to 55) and late (d 81 to 111) gestation, as-fed basis¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td>Early/late</td>
<td>Early</td>
</tr>
<tr>
<td>Corn</td>
<td>70.40</td>
<td>22.80</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>8.56</td>
<td>23.81</td>
</tr>
<tr>
<td>Sugar</td>
<td>8.56</td>
<td>23.81</td>
</tr>
<tr>
<td>Solka-Floc²</td>
<td>3.00</td>
<td>13.00</td>
</tr>
<tr>
<td>Canola oil</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Celite</td>
<td>0.07</td>
<td>0.14</td>
</tr>
<tr>
<td>L-His</td>
<td>0.24</td>
<td>0.28</td>
</tr>
<tr>
<td>L-Leu</td>
<td>—</td>
<td>0.24</td>
</tr>
<tr>
<td>L-Lys-HCl</td>
<td>0.79</td>
<td>0.72</td>
</tr>
<tr>
<td>DL-Met</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>L-Cys-HCl</td>
<td>0.17</td>
<td>0.28</td>
</tr>
<tr>
<td>L-Phe</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>L-Tyr</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>L-Trp</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>L-Val</td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>L-Glu</td>
<td>2.00</td>
<td>6.30</td>
</tr>
<tr>
<td>Vitamin-mineral premix³</td>
<td>3.80</td>
<td>—</td>
</tr>
<tr>
<td>Mineral premix⁴</td>
<td>—</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin premix⁵</td>
<td>—</td>
<td>0.70</td>
</tr>
<tr>
<td>Choline chloride⁶</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.50</td>
<td>2.40</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.16</td>
<td>0.42</td>
</tr>
<tr>
<td>KHCO₃</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Limestone</td>
<td>—</td>
<td>1.10</td>
</tr>
<tr>
<td>KCl</td>
<td>—</td>
<td>0.35</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Energy and nutrient contents

| Thr, g/kg (analyzed)        | 1.8              | 0.9              | 2.6             |
| Lys, g/kg (analyzed)        | 8.1              | 6.2              | 9.4             |
| ME, MJ/kg (calculated)      | 13.82            | 13.96            | 14.81           |
| CP, % (calculated)          | 9.66             | 8.14             | 10.67           |
| Ca, % (calculated)          | 0.97             | 0.97             | 0.97            |
| Available P, % (calculated) | 0.45             | 0.45             | 0.45            |

¹Daily feed intake set to achieve recommended intake based on sow BW and backfat at breeding (Aherne and Foxcroft, 2000). Feed intake was 2.4 ± 0.1 kg.

²Provided per kilogram of diet: 82 g of Ca; 32 g of P; 18 g of Na; 4 g of Mg; 23 mg of Cu as CuSO₄; 267 mg of Fe as ferrous sulfate; 61 mg of Mn as MnO; 0.3 mg of Se; 132 mg of Zn as ZnO; 0.4 mg of I as Ca(IO₃)₂; 0.2 mg of biotin; 7 mg of riboflavin; 33 µg of vitamin B₁₂; 11,400 IU of vitamin A; 50 IU of vitamin E; 1,330 IU of vitamin D₃; 1.6 mg of vitamin K; 494 mg of choline; 3 mg of folacin; 36 mg of niacin; and 24 mg of pantethenic acid (Consultant Feeds, Calmar, Alberta, Canada).

³Provided per kilogram of diet: 50 mg of Cu as CuSO₄; 75 mg of Fe as ferrous sulfate; 25 mg of Mn as MnO; 0.3 mg of Se; 125 mg of Zn as ZnO; and 0.5 mg of I as Ca(IO₃)₂ (DSM Nutritional Products, Hay River, Alberta, Canada).

⁴Provided per kilogram of diet: 10,500 IU of vitamin A; 1,050 IU of vitamin D₃; 70 IU of vitamin E; 5.6 mg of vitamin K; 3.5 mg of thiamine; 7 mg of riboflavin; 2.1 mg of pyridoxine; 21 µg of vitamin B₁₂; 50 µg of niacin; 21 mg of pantothenic acid; 3.5 mg of folic acid; and 0.4 g of biotin (DSM Nutritional Products).

⁵Provided per kilogram of mineral mixture: 731 and 1,462 mg of choline chloride in Exp. 1 and 2, respectively.
door for the sows to enter and exit the chamber and a plexiglass side window. Access to the sows was through a removable piece of plexiglass on top of and near the front of the chamber.

Before entering the respiration chambers, the vascular port was accessed using a 22-gauge needle set (Softee right-angle Huber needle set, Instech Solomon) and a 1.5-m extension set (Smiths Medical Canada Ltd., Markham, Ontario, Canada). The extension set was externalized from the chamber to facilitate blood sampling during collection of expired air. Sows were placed in the respiration chambers 30 min before the beginning of the collection of expired air to allow the air in the chamber to equilibrate with the ventilating air stream. Each respiration chamber was fitted with a 10-cm-diameter capped polyvinyl chloride tube, which allowed feed to be dropped into the feeder and a nipple drinker for ad libitum access to fresh water. The chambers were designed with 2 air inlets, each consisting of 2.5-cm diameter acrylonitrile butadiene styrene pipe spanning the length of the chamber and 1.27-cm holes drilled approximately every 30 cm and capped at the opposite end. Ambient air was drawn through the chambers by rotary vane pumps (Gast Model 1023, Gast Manufacturing Inc., Benton Harbor, MI). Air flow was set at 240 L/min to maintain CO₂ concentration below 1.0%.

The CO₂ in ambient air was measured using nondispersive near-infrared analyzers (Qubit Systems, Kingston, Ontario, Canada). Expired air was collected in 30-min intervals into 11 mL of 1 N NaOH solution. Background ¹³CO₂ enrichment was measured for three 30-min periods before administration of the isotope (Figure 1).

Expired air samples were analyzed for ¹³CO₂ according by following the procedure reported by el-Khoury et al. (1994). Expired CO₂, which was trapped in 1 N NaOH solution in the form of Na₂CO₃, was reacted with H₃PO₄ to release the free CO₂ gas. The ¹³CO₂ enrichment in the gas was then measured by a continuous flow, dual-inlet isotope ratio mass spectrometer (CF-IRMS 20/20 isotope analyzer, PDZ Europa Ltd., Cheshire, UK). Each set of 8 samples (2 baseline and 6 plateau) was separated by reference samples (5% CO₂), which were previously calibrated to an international reference standard (NBS-20, National Institute for Standards and Technology, Gaithersburg, MD). Expired air ¹³CO₂ enrichment was calculated as the difference in isotopic abundance at plateau and natural (baseline) abundance and was expressed as atom percent excess (APE). Plateau ¹³CO₂ enrichment value for each sow/diet combination was determined as the data points where the linear regression of enrichment within collection period was not different from zero. This was achieved for all studies within 120 min from the start of isotope administration. The L-[1-¹³C]Phe oxidation rates were expressed as a percentage of the infused dosage. Blood samples (5 mL) were collected in heparinized Vacutainer tubes at 30-min intervals immediately before feeding during the five 0.5-h collection period (Figure 1). Blood samples were centrifuged at 1,500 × g for 15 min at room temperature for separation of plasma. Reverse phase HPLC (Waters, Milipore, Mississauga, Ontario, Canada) with the use of phenylisothiocyanate derivatives was used to measure plasma AA (Bidlingmeyer et al., 1984).

The L-[1-¹³C]Phe oxidation rates (OXdPhe) were calculated according to the equation
Threonine requirement of gestating sows


\[ \text{OX_{Phe}} = F^{13}\text{CO}_2 / \text{Phe}_{\text{inf}} \]

where \( F^{13}\text{CO}_2 \) = the rate of \(^{13}\text{CO}_2 \) released by Phe tracer oxidation (mmol \(^{13}\text{CO}_2 /30\) min), and \( \text{Phe}_{\text{inf}} \) = rate of \( \text{L-}[1-^{13}\text{C}]\text{Phe} \) tracer administered (mmol \(^{13}\text{C-Phe}/30\) min). The \( F^{13}\text{CO}_2 \) was calculated according to the equation (Matthews et al., 1980):

\[ F^{13}\text{CO}_2 = (FCO_2 \times ECO_2 \times 30 \times 42.3)/(100 \times 0.82), \]

where \( FCO_2 \) = rate of \( \text{CO}_2 \) production (mL/min) and \( ECO_2 = \text{CO}_2 \) enrichment in expired air at isotopic steady state (APE). The constants 30 min and 42.3 mmol/mL convert \( FCO_2 \) to mmol/30 min, and the factor 100 changes APE to a fraction. The constant 0.82 accounts for \(^{13}\text{CO}_2 \) retained in the body because of bicarbonate fixation (Mochn et al., 2004b).

The formula by Brouwer (1965) was used to calculate heat production from indirect calorimetry:

\[
\text{Heat production} = 1.44 \times [(16.18 \times V_{O_2}) + (5.02 \times V_{CO_2}) - (2.17 \times V_{CH_4})],
\]

where \( V_{O_2}, V_{CO_2}, \) and \( V_{CH_4} \) are the volumes (L) of \( O_2, CO_2, \) and \( CH_4 \) produced, respectively. The formula was abbreviated by omitting the urinary \( N \). The effect of ignoring the urinary \( N \) (i.e., protein metabolism) is 1% for every 12.3% of the total energy that was derived from protein (Weir, 1949).

**Statistical Analysis**

Daily feed intake was not equal among sows; therefore, daily Thr intake was expressed as g Thr/d rather than percent dietary Thr. Data used to determine Thr requirement in EG and LG (plasma AA and IAAO) from each experiment were analyzed separately. Data were initially analyzed using linear, quadratic, and cubic regression to determine the model of best fit. Nonlinear regression analysis was conducted on data where the model of best fit was quadratic or cubic. The NLIN procedure (SAS Inst. Inc., Cary, NC) was used to determine the broken-line regression model of best fit (i.e., broken line, quadratic broken line, or 2-slope broken line) based on fit statistics and gradient values for each variable (i.e., the breakpoint, the asymptote, and slopes of the line segments; Robbins et al., 2006). Based on the broken-line model of best fit, the NLMixed procedure was used to establish the Thr requirement in EG and LG to account for the random effect of sow (Robbins et al., 2006). The method described by Fadel (2004) was used to determine the starting estimates for the breakpoint and the slope of the line below the breakpoint for the NLMixed analysis.

Data were tested for outliers using the REG procedure of SAS. Correlation analysis, using the CORR procedure of SAS, was used to identify covariables affecting the dependent variable response (i.e., \( \text{L-[L-^{13}\text{C}]Phe} \) Phe oxidation and plasma AA) irrespective of the effect of dietary Thr level. Sow BW, maternal gestation BW gain, parity (Exp. 2), litter weight, litter size, and room temperature were tested as potential covariables. Stepwise regression was used to ensure all covariables identified entered the model at no greater than \( P = 0.10 \). In Exp. 1, room temperature was included in the model for \( \text{L-[L-^{13}\text{C}]Phe} \) oxidation for the EG analysis only. Room temperature varied during this period because of mechanical problems. In both EG and LG, BW was included in the model for plasma Thr. No other covariables reached significance. The difference in Thr requirement between EG and LG was evaluated by a paired \( t \)-test. The effect of parity was tested in Exp. 2. For all analyses, \( P \leq 0.05 \) and \( P \leq 0.10 \) were considered as significant and a tendency, respectively.

**RESULTS**

Maternal BW gain was 42.2 ± 7.8, 42.3 ± 2.9, and 32.1 ± 1.8 in the second, third, and fourth parity sows, respectively. In Exp. 1, average litter size was 12.6 but ranged from 8 to 18, and the litter weight was 19.3 ± 1.0 kg (Table 2). In Exp. 2, average litter size was 14.0 but ranged from 9 to 17 and the litter weight was 21.3 ± 1.0 kg. The increase in energy intake in LG in Exp. 2 seemed to prevent the negative energy balance observed in LG in Exp. 1 where heat production was numerically greater than energy intake.

**Exp. 1**

Oxidation of excess indispensable AA, as represented by oxidation of \( \text{L-[L-^{13}\text{C}]Phe} \), was reduced as daily Thr intake approached the requirement (Figure 2). There was no further change in oxidation of \( \text{L-[L-^{13}\text{C}]Phe} \) with increased Thr intake after the daily requirement for Thr was satisfied as defined by the breakpoint. Breakpoint analysis determined a daily Thr requirement of 6.1 ± 0.4 g/d in EG (\( R^2 = 0.59 \)) and 13.6 ± 3.6 g/d in LG (\( R^2 = 0.60 \)) based on indicator oxidation (Figure 2).

Plasma Thr concentration remained constant, then increased rapidly as Thr intake increased above the requirement in EG. A breakpoint was determined at 7.0 ± 0.5 g of Thr/d (\( R^2 = 0.90 \), Figure 2). In LG, plasma Thr concentration increased linearly with increasing daily Thr intake (\( R^2 = 0.83 \)); thus, a breakpoint was not established (Figure 2). Plasma Phe was not different across Thr intakes in EG or LG. Plasma concentrations of other essential AA were not affected by dietary Thr (data not shown). The requirement for Thr based on IAAO tended to be greater in LG compared with EG (\( P = 0.06 \)).

**Exp. 2**

Similar to the Exp. 1, oxidation of excess \( \text{L-[L-^{13}\text{C}]Phe} \) was reduced as daily Thr intake approached the
requirement as defined by the breakpoint. Breakpoint analysis determined a daily Thr requirement of 5.0 ± 1.0 g/d in EG (R^2 = 0.71) and 12.3 ± 2.3 g/d in LG (R^2 = 0.58) based on indicator oxidation (Figure 3). No further reduction in L-[1-13C]Phe oxidation occurred at dietary Thr intakes above requirement.

In both EG and LG, plasma Thr concentrations remained constant, followed by a large increase at Thr intakes above the requirement. Breakpoint analysis determined a breakpoint at 3.9 ± 0.5 g/d (R^2 = 0.93) in EG and 10.5 ± 2.8 g/d (R^2 = 0.67) in LG (Figure 3). Plasma Phe was not different across Thr intakes in EG or LG. There was no effect of dietary Thr intake on plasma concentrations of other essential AA (data not shown). There was no effect of parity on Thr requirement. The requirement for Thr in LG based on IAAO was greater than the Thr requirement in EG (P < 0.01).

**DISCUSSION**

Based on IAAO, the Thr requirement of gestating sows was in the range of 5.0 and 6.0 g/d in EG and in the range of 12.3 to 13.6 g/d in LG. The greater BW gain in LG compared with EG likely played a role in the greater requirement for Thr in LG compared with EG. The sows were in a negative energy balance in LG in Exp. 1, which may have affected the response to increasing daily Thr intake. The greater daily energy intake in LG in Exp. 2 prevented the negative energy balance observed in Exp. 1, although there was only a minor numerical difference between the estimated Thr requirements for LG in Exp. 1 and 2.

The constant plasma Phe concentration observed in the current study at all daily Thr intakes indicates that in the presence of deficient dietary Thr, all excess Phe was oxidized and the isotopic label expired as 13CO_2. There was no change in the other plasma free essential AA (except Thr) in response to variations in Thr intake. Zhao et al. (1986) also found no change in plasma concentration of other free AA with increasing dietary Thr intake in adult men.

The requirement for Thr was up to 40% less (approximately 6 vs. 10 g/d) from d 35 to 53 of gestation and up to 30% greater (approximately 13 vs. 10 g/d) from d 92 to 110 of gestation compared with the NRC (1998) recommendation for sows of similar BW, expected growth in gestation, and litter size. This large discrepancy with the NRC (1998) recommendation was not expected; thus, the range of daily Thr intakes utilized in Exp. 1 were inadequate to confidently establish the Thr requirement. In EG, the lower limit of daily Thr intakes was marginally less than the established Thr requirement, whereas in LG, the upper limit of daily Thr intakes was marginally greater than the established Thr requirement. However, the results of Exp. 2, obtained with a wider range of Thr intakes, confirmed the 2-fold increase in the Thr requirement in LG compared with EG established in Exp. 1. This is in contrast to Dourmad and Étienne (2002) who did not find an effect of gestation stage on Lys and Thr requirements. However, our findings are in agreement with recent results (Srichana, 2006; Samuel et al., 2010) showing that the Lys requirement of sows in early and mid gestation (30 to 80 d) is less than in late gestation (90 to 100 d). This experimental evidence is in agreement with recently published recommendations for sow feeding (GfE, 2008; Kim et al., 2009) that indicate increased AA requirements in LG compared with EG based on models of gestational growth.

Preliminary results from our group (Samuel et al., 2010) using the same genetics as the current study indicate that the requirement for Lys in early gestation is 13.1 and 8.2 g/d in second and third parity sows, respectively. The Lys requirement in late gestation increased to 18.7 and 13.0 g/d in second and third parity sows, respectively. Based on the current results and those of Srichana (2006) and Samuel et al. (2010), calculating a Lys:Thr ratio using the NRC (1998) Lys recommendation would be inappropriate. However, using
the preliminary results of Samuel et al. (2010) and the current study, the Lys:Thr ratio for second parity sows would be 1:0.47 and 1:0.72 in EG and LG, respectively, and 1:0.61 and 1:0.95 for third parity sows in EG and LG, respectively. The greater Thr relative to Lys with increasing parity implies that maintenance of the sow plays a greater role, and lean growth of the sow plays a lesser role in the requirement for AA as the sow age increases. The greater Thr relative to Lys with increasing parity is in agreement with GfE (2008), although the GfE (2008) did not indicate a difference in Thr requirement within parity (parity 2: 1:0.70 and 1:0.66 in EG and LG, respectively; parity 3: 1:0.76 and 1:0.68 in EG and LG, respectively).

Figure 2. The response of sows to increasing dietary Thr intake based on l-[1-13C]Phe oxidation and plasma Thr (A: early gestation; B: late gestation) in Exp. 1. The breakpoint, as determined by broken-line nonlinear regression, represents the daily Thr requirement (shown as arrows within each panel). The Thr requirement was 6.1 ± 0.4 g/d (R² = 0.59) and 7.0 ± 0.5 g/d (R² = 0.90) in early gestation based on indicator oxidation (shown as closed boxes) and plasma Thr (shown as +), respectively. The Thr requirement was 13.6 ± 3.6 g/d (R² = 0.60) in late gestation based on indicator oxidation. Plasma Thr increased linearly (−0.1 + 0.03*X, R² = 0.83, where X = daily Thr intake, g/d) in late gestation; thus, no breakpoint was established. Color version available in the online PDF.
The GfE (2008) suggested a similar Thr requirement for second parity sows in EG (6.6 g/d on a true ileal digestible basis) but a reduced Thr requirement in LG (9.6 g/d, true ileal digestible basis) compared with the results of the current study. Conversely, Kim et al. (2009) suggested substantially smaller Thr requirements for early and late gestation of approximately 3.3 and 6.1 g/d, respectively, on a true ileal digestible basis.

Figure 3. The response of sows to increasing dietary Thr intake based on [1-13C]Phe oxidation and plasma Thr (A: early gestation; B: late gestation) in Exp. 2. The breakpoint, as determined by broken-line nonlinear regression, represents the daily Thr requirement (shown as arrows within each panel). The Thr requirement was 5.0 ± 1.0 g/d ($R^2 = 0.71$) and 3.9 ± 0.5 g/d ($R^2 = 0.93$) in early gestation based on indicator oxidation (shown as closed boxes) and plasma Thr (shown as +), respectively. The Thr requirement was 12.3 ± 2.3 g/d ($R^2 = 0.58$) and 10.5 ± 2.8 g/d ($R^2 = 0.67$) in late gestation based on indicator oxidation and plasma Thr, respectively.
Both sets of requirement recommendations (GfE, 2008; Kim et al., 2009) were calculated from the estimated growth of maternal body and conceptus products. However, GfE (2008) included an estimate of efficiency of AA utilization, which was not mentioned by Kim et al. (2009).

The absence of an effect of parity on Thr requirement in Exp. 2 is in contrast to GfE (2008) who suggest decreasing requirements with increasing parity, especially in early gestation. Kim et al. (2009) also suggest split parity feeding, although they do not provide data concerning the change in requirements. The absence of an effect of parity may be caused by the similar gestational BW gain of second and third parity sows and the still substantial maternal gestation BW gain in fourth parity sows. The current results confirm the recent recommendations of GfE (2008) and Kim et al. (2009) in that Thr requirements increase from early to late gestation. However, the present results provided no indication for a decrease in requirements with increasing parity number.

In the current study, the Thr requirement based on plasma AA was less than the requirement based on IAAO except in Exp. 1 where plasma Thr increased linearly with increasing Thr intake in LG. Plasma AA have been shown to correlate poorly with other estimates of requirement or to respond linearly to all treatments (Mitchell et al., 1968; Sohail et al., 1978; Pampuch et al., 2006) and thus are less reliable dependent variables when estimating AA requirements. The quantitative response of plasma AA to changes in dietary AA reflects the complex interaction of the dynamic equilibrium of the plasma AA pool with the tissue AA pool, protein degradation, and labile nonprotein AA sources (Young and Scrimshaw, 1970). The linear decrease in plasma Thr below requirement observed in LG in Exp. 1 may reflect a conservation of the plasma-free Thr pool in response to the increasing fetal demand for nutrients in LG or the increased contribution from protein turnover that occurs with increased protein synthesis in LG.

The current study has several advantages over other similar studies examining AA requirement of sows during gestation. First, the IAAO method requires only 2-d adaption to test AA quantities (Moehn et al., 2004a) so that several amounts of test AA can be studied in a short time period within the same animal. This reduces the effect of the between-subject variation, which is approximately 10% in pigs (Bertolo et al., 2005; Moehn et al., 2008). Furthermore, the IAAO method determines variables specific to AA metabolism being based on the principle that AA are utilized for protein synthesis and any excess AA must be oxidized (Pencharz and Ball, 2003). As the intake of the limiting AA increases, the oxidation of the indicator AA decreases linearly until the requirement for the limiting AA is reached. Increases of the test AA beyond the requirement do not increase protein synthesis further; thus, oxidation of the indicator AA reaches a plateau. The breakpoint is defined as the requirement for the test AA. The IAAO technique has been developed to determine AA requirements in humans (Elango et al., 2007), chickens (Coleman et al., 2003), piglets (Ball and Bayley, 1984), and growing pigs (Moehn et al., 2008) and was chosen as the gold standard by the World Health Organization for determination of AA requirements of humans (WHO, 2007). Second, the requirement was separated into 2 periods, d 25 to 55 to represent EG and d 81 to 111 to represent LG. The summative results of McPherson et al. (2004), Ji et al. (2006), and Kim et al. (2009) clearly indicate that there is an increase in protein accretion from early to late gestation. The current results have further identified the change in daily AA intake required to support the increased protein accretion rate and reduce catabolism of maternal lean tissue in late gestation. Third, the current study utilized 6 different amounts of dietary Thr. Although 4 amounts of dietary nutrient are minimally sufficient, 6 or more allow better fit of the data to a descriptive response curve and thus aid in the objective assessment of requirement (Baker, 1986). Last, the greatest animal-to-animal variability occurs in the upper curvilinear area of a growth curve (Baker, 1986), which is also the area associated with definition of the requirement. Using the IAAO method, the animal-to-animal variation was reduced because each sow received each dietary treatment.

The Thr requirement increased 2-fold in the last third of gestation in multiparous sows (5.0 vs. 12.3 g/d). Therefore, feeding a single amount of AA, as recommended by NRC (1998), throughout gestation results in overfeeding AA in EG and underfeeding AA in LG. Overfeeding AA will increase feed costs and potentially increase environmental contamination through excretion of excess N (Adeola, 1999), whereas underfeeding AA during pregnancy results in breakdown of maternal tissue to support fetal growth and milk production in the next lactation (Clowes et al., 2003). The loss of maternal protein must be reestablished during the subsequent rebreeding and early gestation period. Sow reproductive longevity may be reduced by this continual cycle of protein synthesis and catabolism. Therefore, phase feeding has the potential to improve sow reproductive performance. To develop an appropriate phase feeding program for gestating sows, the requirement for essential AA and the Lys:AA ratios must be established in EG and LG separately.

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