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Dietary supplementation with lysine (protein) in late pregnancy does not enhance mammary development in multiparous sows

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Abstract

This project was conducted to determine if providing standardized ileal digestible (**SID**) Lys at 40% above estimated requirements (NRC, 2012), with the concomitant increased protein intake, from days 90 to 110 of gestation stimulates mammary development in multiparous sows. From day 90 of gestation, Yorkshire × Landrace multiparous sows (parities 2 and 3) were fed 2.6 kg/d of either a conventional diet (**CTL**, control, $n = 17$) providing 14.8 g/d of SID Lys or a diet providing 20.8 g/d of SID Lys via additional soybean meal (**HILYS**, $n = 16$). The diets were isoenergetic. Concentrations of IGF-1, glucose, free fatty acids (**FFA**), urea, and amino acids (**AA**) were measured in jugular blood samples obtained on days 90 and 110 of gestation. Sows were necropsied on day 110 ± 1 of gestation to obtain mammary glands for compositional and histological analyses. Backfat or BW changes of sows during late gestation were unaffected by treatment ($P > 0.10$), as was the case for fetal BW ($P > 0.10$). None of the variables measured in mammary tissue were altered by supplementary Lys ($P > 0.10$). Circulating IGF-1, glucose, and FFA did not differ ($P > 0.10$) between HILYS and CTL sows on day 110 of gestation, whereas concentrations of urea were greater ($P < 0.01$) in HILYS versus CTL gilts. Concentrations of Ile and Thr in plasma were also greater ($P < 0.05$), and those of Glu were lower ($P < 0.01$) in HILYS than CTL sows. These results demonstrate that feeding Lys (via protein) above current NRC recommendations during late gestation does not improve mammary development of multiparous sows. Hence, the use of a two-phase feeding strategy to provide more Lys (protein) to multiparous sows during this period is not necessary.

Lay Summary

Results indicate that there is no advantage in terms of mammary development to feeding late-pregnant multiparous sows with 40% more lysine (via protein) than current recommendations (NRC, 2012). From days 90 to 110 of gestation, multiparous sows (parities 2 and 3) were fed 2.6 kg/d of either a conventional diet providing 14.8 g/d of standardized ileal digestible (SID) lysine or a diet providing 20.8 g/d of SID lysine via the inclusion of additional soybean meal. Diets were isoenergetic. Feeding supplementary SID lysine had no effect on mammary development at the end of gestation. Contrary to our previous report for gilts, mammary gland development is not improved by providing more lysine to multiparous sows in late gestation. Such information is crucial for developing the best feeding strategies to maximize milk yield. The use of a two-phase feeding strategy to provide more lysine (protein) as of day 90 of gestation is not necessary in multiparous sows.

Key words: feeding, gestation, lysine, mammary development, parity, sow

Abbreviations: AA, amino acids; BCAA, branched chain amino acids; BF, backfat thickness; BW, body weight; CTL, control; FFA, free fatty acids; HILYS, high lysine; IGF-1, insulin-like growth factor-1; SID, standardized ileal digestible

Introduction

The long-lasting problem of inadequate sow milk yield to meet the demands of all suckling piglets in a litter remains an ongoing challenge (Farmer, 2022). Given that milk yield of sows depends on the number of milk-synthesizing epithelial cells present in mammary parenchymal tissue at the onset of lactation (Head and Williams, 1991), it is crucial to maximize mammary development. Nutrition of gilts either in the prepubertal period (from 90 d of age onward) or during late gestation (after day 90) can impact mammogenesis (see review by Farmer, 2018). As the first limiting amino acid in swine rations, Lys plays an important role for supporting protein synthesis and we previously determined that supplying Lys above current estimated requirements (NRC, 2012), via the addition of soybean meal, improved mammary development in gilts during late pregnancy (Farmer et al., 2022). Increasing dietary standardized ileal digestible (**SID**) Lys by 40% (from 18.6 to 26.0 g/d via the addition of soybean meal) from days 90 to 110 of gestation significantly increased (42%) mammary parenchymal tissue mass.

Given that the Lys requirement in gestation is greater for primiparous than multiparous sows (Gaillard et al., 2020), the effects of supplementary Lys during late gestation may differ between these two states. Providing additional dietary

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Lys during late gestation and lactation increased litter body weight (BW) gain in both primiparous (Heo et al., 2008) and multiparous sows (Yang et al., 2008). Another study showed that piglet BW gain to weaning was greater for multiparous than primiparous sows, but the response to supplementary dietary Lys in gestation or lactation was similar across parities (Yang et al., 2009). Thomas et al. (2021a) compared the effects of increasing dietary SID Lys (ranging from 11.0 to 18.5 g/d) in gilts or sows throughout gestation, and observed differences due to parity. Feeding 11 g/d of SID Lys was adequate for both gilts and sows; however, providing 18.5 g/d of dietary SID Lys reduced the incidence of stillbirth in sows only. Using modeling to estimate SID Lys requirements for gilts or sows during gestation, these last authors determined that in both cases the requirement increased with advancing gestation, with Lys balance being negative over the last 5 to 10 d of gestation. Thomas et al. (2021b) suggested that the imbalance would be addressed by providing 13.5 g SID Lys/d, instead of 11.0 g/d. However, the impact of dietary Lys supplementation on mammary development or subsequent litter performance was not investigated.

Primiparous sows are still growing during gestation, and differences in metabolism and mammary biology between primiparous and multiparous sows may lead to differential effects of supplementary Lys (via soybean meal) during the critical period of mammogenesis in late gestation. Any difference in responses across parities is likely enhanced in modern sows that are larger and heavier, and that have greater energy requirements for maintenance than older-type sows (Vier et al., 2022). Determining the impact of parity on the beneficial effects of supplementary Lys in late gestation is crucial to developing a best-adapted feeding strategy to enhance mammary development and subsequent lactation performance in swine. Therefore, the goal of the present study was to determine if the previously reported beneficial effect of a 40% increase in SID Lys intake from gestation days 90 to 110 on mammary development of gilts would also occur in multiparous sows.

Materials and Methods

Animals were cared for according to a recommended code of practice (CCAC, 2009) following procedures approved by the institutional animal care committee of the Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada.

Animals and treatments

Thirty-three Yorkshire FAST × Landrace FAST multiparous sows (parities 2 and 3) from the Sherbrooke Research Centre herd were artificially inseminated using pools of semen from Duroc Super Gain Plus boars (Centre d'Insémination Porcine du Québec, Saint-Lambert-de-Lauzon, QC, Canada). Gestating sows were housed in individual pens (1.5 × 2.4 m), where from mating until day 89 of gestation they were fed one daily meal (0800 hours) of a conventional corn-based diet (11.68 MJ/kg DE, 13.99% crude protein, 0.52% SID Lys). The amounts fed were based on a commercial chart using BW and backfat thickness (**BF**) as follows: sows weighing 195 to 209 kg at mating were fed 2.70, 2.55, 2.35, and 2.10 kg, respectively, for a BF of <9, 10 to 12, 13 to 15, or ≥ 16 mm. Sows weighing 210 to 240 kg or more at mating were fed 2.75, 2.60, 2.40, and 2.15 kg for the same BF categories. From day 90 of gestation, all sows were fed 2.6 kg of either a conventional diet (**CTL**, control, $n = 17$) providing 14.8 g/d of SID Lys with all other AA meeting or exceeding NRC (2012) recommendations or a diet providing 20.8 g/d of SID Lys via the inclusion of additional soybean meal (**HILYS**, $n = 16$) with all other AA to Lys ratios meeting or exceeding NRC recommendations. Diets were isoenergetic on a net energy (**NE**) basis (Table 1). Feed samples for AA analyses were collected and pooled within dietary treatment every 2 wk. At mating and on days 90 and 110 of gestation, sows were weighed and their BF was measured ultrasonically (WED-3000, Schenzhen Well D Medical Electronics Co., Guangdong, China) at P2 of the last rib. On days 90 and 110 of gestation, blood samples were collected by jugular venipuncture before the meal (between 0700 and 0800 hours) following 16 h fast. Sows were necropsied on day 110 ± 1 of gestation to obtain mammary glands for various compositional analyses. The uterus was removed and fetuses counted and weighed, and the ovaries weighed and the number of corpora lutea counted.

Blood handling and assays

The concentrations of insulin-like growth factor-1 (**IGF-1**), glucose, free fatty acids (**FFA**), urea, and AA were measured in blood samples. Samples for urea (20 mL) were collected into vacutainer tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ) and held at room temperature for 3 h, stored overnight at 4 °C, centrifuged for 12 min at 1,800 × *g* at 4 °C, before serum was harvested. Blood for IGF-1, FFA, and AA assays (30 mL) was collected in EDTA tubes (Becton Dickinson), held on ice and centrifuged within 20 min for 12 min at 1,800 × *g* at 4 °C, from which plasma was recovered. Lastly, blood samples for glucose analysis (6 mL) were collected into tubes containing 12 mg of potassium oxalate and 15 mg of sodium fluoride to inhibit glycolysis, held on ice and centrifuged within 20 min at 1,800 × *g* for 12 min at 4 °C, and the plasma recovered. Serum and plasma were stored at −20 °C. Concentrations of IGF-1 were measured with a commercial RIA kit for human IGF-1 (ALPCO Diagnostics, Salem, NH) with small modifications as detailed previously (Plante et al., 2011) including validation using a pooled plasma sample from sows. Sensitivity of the assay was 0.10 ng/mL, while the intra- and interassay CVs were 4.99% and 2.45%, respectively. Glucose was measured by an enzymatic colorimetric method (Wako Chemicals, Richmond, VA) that was validated using a plasma pool from gestating sows, where parallelism was 100.8%, and the average mass recovery was 95.5%. Intra- and interassay CVs were 3.19% and 2.71%, respectively. Urea was measured colorimetrically using an autoanalyzer (Auto-Analyser 3; Technicon Instruments Inc., Tarrytown, NY) according to the method of Huntington (1984). Intra- and interassay CVs were 0.85% and 1.10%, respectively. Concentrations of FFA were measured by colorimetry (Wako Chemicals) having intra- and interassay CVs of 0.67% and 2.04%, respectively.

Plasma-free AA concentrations were analyzed according to the methods of Boogers et al. (2008) and using Ultra Performance Liquid Chromatography and Empower Chromatography Data Software (Waters Corporation, Milford, CT). The experimental diets were analyzed for AA using the performic acid oxidized hydrolysis procedure (Method 994.12; AOAC, 2005) and then were quantified via ion-exchange chromatography with post-column derivatization with ninhydrin according to Llames and Fontaine (1994).

Table 1. Ingredient composition and nutrient contents of experimental diets (as-fed)

Item	CTL[1]	HILYS
Ingredient composition, %		
Corn	61.24	50.71
Wheat	20.00	20.00
Soybean meal, dehulled, solvent extracted	15.00	24.50
Soybean oil	0.38	1.43
Di-calcium phosphate	1.20	1.10
Limestone	1.58	1.55
Sodium chloride	0.30	0.30
Vitamin and mineral premix[2]	0.20	0.20
DL-Met	0	0.06
L-Thr	0.02	0.07
Choline chloride 70%[3]	0.08	0.08
Total	100.00	100.00
Calculated nutrient contents[4]		
NE, kcal/kg	2,500	2,500
Crude protein, %	14.41	18.15
SID Lys, %[5]	0.57	0.80
SID Arg, %	0.78	1.05
SID His, %	0.35	0.43
SID Ile, %	0.49	0.64
SID Leu, %	1.11	1.32
SID Met, %	0.22	0.32
SID Met + Cys, %	0.46	0.60
SID Phe, %	0.61	0.78
SID Phe + Tyr, %	1.00	1.28
SID Thr, %	0.45	0.63
SID Trp, %	0.14	0.20
SID Val, %	0.56	0.72
Total Ca, %	0.87	0.87
STTD phosphorus, %	0.37	0.37
Analyzed nutrient contents		
Crude protein, %	14.12	15.76
Lys, %	0.68 (0.67)[5]	0.88 (0.92)
Arg, %	0.71 (0.85)	1.00 (1.14)
His, %	0.34 (0.40)	0.45 (0.49)
Ile, %	0.53 (0.56)	0.68 (0.73)
Leu, %	1.21 (1.27)	1.43 (1.51)
Met, %	0.26 (0.25)	0.34 (0.36)
Met + Cys, %	0.50 (0.53)	0.59 (0.68)
Phe, %	0.61 (0.70)	0.80 (0.89)
Phe + Tyr, %	0.93 (1.16)	1.26 (1.47)
Thr, %	0.48 (0.52)	0.65 (0.74)
Trp, %	—(0.16)	—(0.22)
Val, %	0.60 (0.66)	0.72 (0.83)
Ca, %	0.73	0.83
Phosphorus, %	0.49	0.56

[1]CTL: control, Lys provided at estimated requirements for multiparous sows between days 90 and 114 of gestation (NRC, 2012); HILYS: Lys provided 1.4 × above estimated requirements via the addition of soybean meal.
[2]Provided the following amounts of vitamins and trace minerals per kilogram of diet: vitamin A, 1,000 IU; vitamin D_3, 1,500 IU; vitamin E, 40 IU; vitamin K, 2.5 mg; vitamin B_{12}, 20 µg; thiamine, 0.97 mg; riboflavin, 4 mg; D-pantothenic acid, 20 mg; niacin, 20 mg; folic acid, 4.9 mg; biotin, 0.40 mg; pyridoxine, 3.0 mg; Fe, 80 mg as $Fe_2(SO_4)_3$; Zn, 101 mg as ZnO; Mn, 40 mg as MnO_2; Cu, 15 mg as $CuSO_4$; Se, 0.30 mg as Na_2SeO_3; Cr, 0.20 mg as $C_9H_{15}CrO_6$ (Nutreco Canada, Inc., Saint-Hyacinthe, QC, Canada).
[3]Choline chloride (70%; Jefo, Saint-Hyacinthe, QC, Canada).
[4]Based on nutrient concentrations in feed ingredients according to the NRC (2012).
[5]Calculated total amino acid contents are shown in parentheses.

Mammary gland measurements

At necropsy, mammary glands from one side of the udder were excised to assess mammary composition. Glands were frozen and stored at −20 °C, then were cut transversally into 2-cm slices while frozen, prior to being stored again at −20 °C. Each slice was later trimmed of skin and teats at 4 °C, and the mammary parenchyma was dissected from surrounding adipose tissue (i.e., extraparenchymal tissue). Parenchyma from all dissected and sliced glands was homogenized and a representative sample used to determine composition by chemical analysis. The RNA content of parenchymal tissue was measured by ultraviolet spectrophotometry (Volkin and Cohn, 1954), and the DNA content was evaluated by fluorimetry (Labarca and Paigen, 1980). Parenchymal dry matter (Method 950.46; AOAC, 2005), protein (Method 928.08; AOAC, 2005), and lipid content (Method 991.36, AOAC, 2005) were also determined. Both RNA and DNA contents are reported on a dry matter basis. The fifth gland of the contralateral row of mammary glands was sampled for histology and immunohistochemistry. Samples were fixed in 4% neutral buffered paraformaldehyde for 24 h at 4 °C, and then washed twice with 70% ethanol, in which they were stored prior to embedding in paraffin.

Histology and immunohistochemistry

Paraffin-embedded samples were sectioned at 4.5 µm. Cell proliferation was determined from immunohistochemical localization of Ki67 in sections that were rehydrated and pretreated with 0.3% Triton-X in PBS before steaming in Tris buffer (pH 9) and blocking for endogenous biotin. Sections were blocked with 10% horse serum and then incubated overnight at 4 °C with a biotinylated rat monoclonal anti-Ki67 antibody (RRID:AB_2572794; 1:200; Thermo Fisher Scientific, Waltham, MA) and a mouse monoclonal antibody against β-catenin (RRID:AB_1030943; 1:200: Cell Signaling, Danvers, MA). Sections were then rinsed in 0.05% PBS-Tween20 and incubated with both an Alexa Fluor 488-conjugated anti-mouse secondary antibody to localize β-catenin (RRID:AB_2340694; 1:200) and an Alexa Fluor 647 streptavidin-conjugated donkey anti-rat secondary antibody (RRID:AB_2340694; 1:500) for 1 h at room

temperature. All sections were counterstained with DAPI (1:1,000). For each section that represented a sow, $n = 5$ random images were captured at the appropriate wavelengths using a 20× objective on a BX51 microscope using a QICAM Fast 1394 camera. The incidence of Ki67-positive nuclei in the β-catenin positive epithelium in each field was quantified using an in-house macro in FIJI (https://imagej.net/software/fiji/) utilizing the StarDist plugin (Schmidt et al., 2018), and expressed relative to the associated number of DAPI-positive epithelial nuclei to determine the percent labeling index. The alveolar perimeter was determined by manually tracing the apical membrane of all complete alveoli within a composite fluorescent image, as calibrated against a stage micrometer.

Statistical analyses

Statistical analyses were performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The univariate model used for mammary gland composition, gene expression, and immunohistochemistry, as well as ovarian and fetal data included the effect of treatment, with the residual error being the error term used to test for main effects of treatment. An ANOVA with heterogeneous variances was used when necessary. The ANOVA for weight of fetuses included litter size as a covariate. Repeated measures ANOVAs with the factors of treatment (the error term being sow within treatment) and day of gestation (the residual error being the error term), and the treatment by day interaction, were also performed on BW, BF, and blood data. Separate analyses of variance for each day were also carried out on these variables. Data in tables are presented as least squares means ± maximal SEM.

Results

There were no differences due to treatment for BW or backfat thickness of sows on any day (Table 2), nor were there any treatment effects on changes in BW or backfat thickness over the experimental period. The total number of corpora lutea was not affected by treatment, with values of 27.5 and 26.4 ± 1.2 for CTL and HILYS sows, respectively. The combined weight of both ovaries tended to be greater (43.4 vs 37.2 ± 2.3 g, $P = 0.065$) in HILYS vs CTL sows. Litter size on day 110 of gestation was similar across treatments (16.7 vs 18.1 ± 1.03 for CTL and HILYS sows, respectively), while weight of fetuses corrected for litter size was also unaffected by treatment (1.26 vs 1.23 ± 0.04 kg for CTL and HILYS, respectively).

Circulating concentrations of IGF-1, glucose, FFA, and urea in sows are shown in Table 3. The only variable affected by treatment was urea, with concentrations being greater in HILYS than CTL sows on day 110 of gestation ($P < 0.01$). There was a tendency for FFA concentrations to be greater on day 110 than on day 90 of gestation ($P = 0.091$). Regarding AA concentrations in blood (Table 4), both the essential AA, Ile ($P < 0.05$) and Thr ($P \leq 0.01$), were greater in HILYS than CTL sows on day 110 of gestation, whereas the opposite was true for the non-essential AA Glu ($P < 0.01$). Seven AA had levels that were significantly different by day of gestation. Concentrations of the essential AA Ile, Met, and Phe, and the non-essential AA Ala and Tyr were greater on day 110 than 90 of gestation ($P < 0.05$). On the other hand, concentrations of the essential AA Lys and Val ($P < 0.05$) decreased between days 90 and 110 of gestation. Data on mammary gland composition and immunohistochemical variables are presented in Table 5, where none were affected by treatment.

Discussion

Current results demonstrate that, contrary to our previous findings in primiparous sows (Farmer et al., 2022), mammary development in multiparous sows does not benefit from feeding SID Lys 40% above estimated requirements (NRC, 2012) between days 90 and 110 of gestation. This difference in the mammary response to supplementary Lys feeding in late gestation during various parities is a novel finding that highlights how the nutritional requirements for mammogenesis differ between the development of mammary tissue in the first and subsequent pregnancies. On the one hand, this finding shows that the previously reported increase in growth rate of suckling piglets when 40% more SID Lys was fed to late-pregnant multiparous sows (Che et al., 2019) was not due to improved mammary development. On the other hand, it is conceivable

Table 2. Body weight (BW) and backfat thickness of sows fed a control diet (CTL, $n = 17$) or a lysine-supplemented diet (HILYS, $n = 16$) from days 90 to 110 of gestation

Variable measured	Treatment		SEM[1]	*P*-value
	CTL	HILYS		
BW[2], kg				
Mating	229.4	229.1	3.5	0.96
Day 90 of gestation	267.4	267.0	3.0	0.93
Day 110 of gestation	284.0	285.1	3.2	0.80
Gain from days 90 to 110	16.6	18.1	0.8	0.19
Backfat thickness[2], mm				
Mating	17.3	16.4	1.2	0.60
Day 90 of gestation	18.7	18.6	1.1	0.89
Day 110 of gestation	19.5	18.7	1.2	0.64
Gain from days 90 to 110	0.7	0.2	0.3	0.16

[1]Maximum value for the standard error of the mean (SEM).
[2]Day effect ($P < 0.001$).

Table 3. Circulating concentrations of insulin-like growth factor-1 (IGF-1), glucose, free fatty acids (FFA), and urea for sows fed a control diet (CTL, *n* = 17) or a lysine-supplemented diet (HILYS, *n* = 16) from days 90 to 110 of gestation

Variable measured	Treatment		SEM[1]	*P*-value
	CTL	HILYS		
IGF-1, ng/mL				
Day 90 of gestation	30.0	24.7	2.4	0.12
Day 110 of gestation	27.2	24.1	2.1	0.29
Glucose, mmol/L				
Day 90 of gestation	3.33	3.24	0.07	0.37
Day 110 of gestation	3.34	3.31	0.08	0.74
FFA[2], µEq/L				
Day 90 of gestation	149.1	168.3	19.6	0.48
Day 110 of gestation	214.1	183.1	28.8	0.45
Urea[3], mmol/L				
Day 90 of gestation	7.12	7.65	0.32	0.24
Day 110 of gestation	6.68	8.01	0.28	0.002

[1]Maximum value for the standard error of the mean (SEM).
[2]Tendency for a day effect ($P < 0.10$).
[3]Tendency for treatment by day interaction ($P < 0.10$).

that the requirements for development of mammary tissue during the first gestation are greater given that gilts are still growing and because there might be less parenchymal tissue present at day 90 of gestation (onset of treatment) compared with multiparous sows. Galiot et al. (2023) also noted that precision feeding, to increase feed intake during late gestation, had a positive impact on BW gain of gilts, but had less of an effect during their second parity. In the previous study (Farmer et al., 2022), the fact that gilts were growing during their first gestation was evidenced by the increased BW gain between days 90 and 110 of gestation when supplementary Lys was provided, on the other hand, no such increase in BW was seen in multiparous sows receiving the same treatment (present study). The weights of both extraparenchymal and parenchymal mammary tissues in CTL multiparous sows on day 110 of gestation in the current study were greater than in CTL gilts in a previous study (Farmer et al., 2022) by ~40% (42.3% for extraparenchyma and 39.5% for parenchyma), which explains the greater milk yield in multiparous compared with primiparous sows (review by King, 2000). The greater amount of parenchymal tissue at the end of gestation in multiparous sows likely reflects the presence of more parenchyma at mating postweaning compared to when mating occurred at puberty. Assuming that was the case, the Lys requirements for mammary growth in late gestation would be reduced in multiparous sows compared with gilts, supporting the absence of any beneficial effect of additional Lys on mammary development in the current study.

Ford et al. (2003) studied mammary involution postweaning and described the loss of approximately two-thirds of parenchymal mass. These authors suggested that a greater amount of tissue post-involution could benefit redevelopment of mammary tissue in the subsequent gestation. Accordingly, Nielsen et al. (2001) reported that the amount of mammary tissue present at the end of lactation is significantly greater in multiparous than primiparous sows. Even though factors during lactation, such as suckling intensity (review by Farmer, 2019), may affect mammary development by the end of lactation, it is likely that this greater amount of mammary tissue at weaning also reflects greater mammary mass at the end of the previous gestation. On the other hand, Hurley (2019) suggested that suckling-stimulated mammary gland growth during lactation can partially overcome prepartum effects on mammary development. The mass of parenchymal DNA doubled throughout lactation in primiparous sows (Kim et al., 1999), yet the extent of that increase has not been documented in multiparous sows. Nevertheless, our current findings clearly indicate a greater opportunity to stimulate mammary development in the first versus subsequent pregnancies.

An effect of parity on the response of sows to dietary treatment in late gestation was also recorded by Wijesiriwardana et al. (2021), who observed a tendency for a greater effect of supplemental fiber and branched chain AA (BCAA) in late gestation (as of day 110) in primiparous versus multiparous sows when evaluated as piglet growth until weaning. One of their hypotheses was that primiparous sows undergo more intense mammary growth during gestation compared to multiparous sows, supporting the proposal that consumption of additional BCAA could benefit extra protein deposition into mammary tissue. Those authors also suggested that the energy requirements for maintenance versus reproductive functions need to be re-evaluated in gestating gilts and multiparous sows because of the heavier BW of current genetic lines (Vier et al., 2022). Along these lines, the effect of parity on Lys requirement of lactating sows was recently demonstrated (Gaillard et al., 2020). Our present findings indicate that an increase in Lys requirement in primiparous compared with multiparous sows also applies to late gestation with respect to mammary development. Thomas et al. (2021a) recently evaluated the Lys requirement (ranging from 11 to 18.5 g/d SID Lys) during pregnancy for modern sow genotypes. Those authors only recorded minimal effects of SID Lys supplementation on piglet birth weight and reproductive performance in both primiparous and multiparous sows, and concluded that 11 g/d of SID Lys is adequate during gestation. However, those authors did not assess piglet growth in the subsequent lactation period, so there was no indication

Table 4. Circulating concentrations of essential and non-essential AA in sows fed a control diet (CTL, *n* = 17) or a lysine-supplemented diet (HILYS, *n* = 16) from days 90 to 110 of gestation

Variable measured	Treatment		SEM[1]	*P*-value
	CTL	HILYS		
Essential AA, μmol/L				
Arg				
Day 90	159	150	4.8	0.22
Day 110	162	163	7.4	0.94
His[2]				
Day 90	113	111	3.5	0.68
Day 110	105	115	3.6	0.06
Ile[3,4]				
Day 90	94	94	3.3	0.96
Day 110	100	111	3.2	0.02
Leu				
Day 90	166	161	5.1	0.44
Day 110	166	155	10.2	0.42
Lys[4]				
Day 90	115	123	5.0	0.23
Day 110	99.6	105	7.0	0.56
Met[4]				
Day 90	44	42	1.3	0.37
Day 110	46	47	1.7	0.69
Phe[4]				
Day 90	85	83	2.7	0.62
Day 110	88	91	3.0	0.44
Thr[2]				
Day 90	110	114	4.6	0.47
Day 110	112	136	6.5	0.01
Trp[2]				
Day 90	73	67	3.1	0.17
Day 110	67	72	3.4	0.25
Val[4]				
Day 90	220	221	7.1	0.92
Day 110	183	198	7.7	0.17
Non-essential AA, μmol/L				
Ala[4]				
Day 90	337	306	17.0	0.20
Day 110	423	407	19.0	0.56
Asp[5]				
Day 90	11	14	2.4	0.32
Day 110	9	9	0.8	0.50
Cys[3]				
Day 90	3	2	0.4	0.05
Day 110	3	4	0.5	0.29
Glu[3]				
Day 90	198	190	9.7	0.56
Day 110	219	189	6.9	0.004
Gly[3]				
Day 90	1,042	1,016	31.1	0.55
Day 110	995	1,074	42.8	0.20
Pro[5]				
Day 90	239	238	9.0	0.95
Day 110	222	226	8.5	0.71

Table 4. Continued

Variable measured	Treatment		SEM[1]	*P*-value
	CTL	HILYS		
Ser				
Day 90	135	152	12.0	0.32
Day 110	130	140	5.7	0.23
Tyr[4]				
Day 90	87	89	2.6	0.55
Day 110	103	114	5.2	0.13

[1]Maximum value for the standard error of the mean (SEM).
[2]Treatment by day interaction ($P < 0.05$).
[3]Tendency for a treatment by day interaction ($P < 0.10$).
[4]Day effect ($P < 0.05$).
[5]Tendency for a day effect ($P < 0.10$).

Table 5. Mammary gland composition and immunohistochemical variables for parenchymal tissue from sows fed a control diet (CTL, $n = 17$) or a lysine-supplemented diet (HILYS, $n = 16$) from days 90 to 110 of gestation

Variable measured	Treatment		SEM[1]	*P*-value
	CTL	HILYS		
Extraparenchymal tissue, g	2,407.0	2,348.8	154.6	0.77
Extraparenchyma/BW, g/kg	0.85	0.82	0.05	0.71
Parenchymal tissue, g	2,004.7	2,099.3	163.4	0.65
Parenchyma/BW, g/kg	0.71	0.73	0.05	0.68
Parenchyma/teat, g	258.7	263.3	20.0	0.86
Parenchymal tissue composition				
Dry matter, %	29.0	29.2	1.3	0.89
Fat, % of dry matter	49.4	50.8	2.9	0.69
Fat, g total	289.8	304.3	28.3	0.71
Protein, % of dry matter	44.6	44.3	2.4	0.93
Protein, g total	256.5	260.8	22.3	0.86
DNA, mg/g on dry matter basis	9.35	9.28	0.61	0.94
DNA, g total	5.28	5.54	0.51	0.60
DNA/teat, g	0.68	0.70	0.06	0.88
RNA, mg/g on dry matter basis	12.33	12.27	0.86	0.96
RNA, g total	6.97	7.33	0.68	0.68
RNA/teat, g	0.90	0.92	0.08	0.87
Alveolar circumference, µm	94.0	92.2	5.9	0.73
Ki67-positive epithelial cells, %	5.6	5.0	0.4	0.30

[1]Maximum value for the standard error of the mean (SEM).

of sow milk yield, which is the main variable that would be affected by increased mammary development. On the other hand, those authors also reported that both gilts and sows are in a negative Lys balance over the last 5 to 10 d of gestation when fed 11 g/d of SID Lys (Thomas et al., 2021b). However, the model they used to establish these requirements did not include mammary development per se.

The increased circulating concentrations of urea in HILYS multiparous sows on day 110 of gestation mimics our findings in gilts subjected to the same treatment (Farmer et al., 2022) and likely reflects the greater intake of dietary protein. This response was unlikely due to enhanced use of protein as an energy source considering there were no differences in BW and body condition in sows across treatments. Increased circulating urea concentrations in gestating sows fed supplementary Lys were also recorded by Che et al. (2019) and Hong et al. (2020). The greater concentrations of Ile and Thr in the blood of HILYS sows indicate an excess of these AA relative to requirements. No such increases were observed in gilts after a similar treatment, further corroborating a difference in protein requirement during gestation across parities (NRC, 2012). On the other hand, the lower Glu concentrations in HILYS vs CTL sows were surprising, but were also seen in gilts (Farmer et al., 2022).

In conclusion, contrary to what was previously reported in gilts, there is no advantage for development of mammary tissue or fetal growth to providing 40% more dietary Lys to multiparous sows during late gestation. Hence, the use of a two-phase feeding strategy with increased Lys as of day 90 of gestation is not necessary to stimulate mammogenesis in multiparous sows.

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Conflict of Interest Statement

All authors declare that they have no conflict of interest.

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