Title

Dietary supplementation with lysine (protein) stimulates mammary development in late pregnant gilts.

Journal

Journal of Animal Science, 100(5)

ISSN

0021-8812

Authors

Farmer, Chantal
Palin, Marie-France
Hovey, Russell C
et al.

Publication Date

2022-05-01

DOI

10.1093/jas/skac051

Peer reviewed

Journal of Animal Science, 2022, **100**, 1–11
https://doi.org/10.1093/jas/skac051
Advance access publication 20 February 2022
Lactation and Mammary Gland Biology

Dietary supplementation with lysine (protein) stimulates mammary development in late pregnant gilts

Chantal Farmer,[†,1] Marie-France Palin,[†] Russell C. Hovey,[‡] Tara D. Falt,[‡] and Lee-Anne Huber[‖]

[†]Agriculture and Agri-Food Canada, Sherbrooke R & D Centre, Sherbrooke, QC J1M 0C8, Canada
[‡]Department of Animal Science, University of California, Davis, CA 95616, USA
[‖]Department of Animal Biosciences, University of Guelph, Guelph, ON N1G 2W1, Canada
[1]Corresponding author: chantal.farmer@agr.gc.ca

Abstract

The goal of this project was to determine if standardized ileal digestible (**SID**) lysine provided at 40% above estimated requirements, with the concomitant increase in protein intake, from days 90 to 110 of gestation would stimulate mammary development in gilts. From day 90 of gestation, Yorkshire × Landrace gilts were fed 2.65 kg of either a conventional diet (**CTL**, control, $n = 19$) providing 18.6 g/d of SID Lys or a diet providing 26.0 g/d of SID Lys via additional soybean meal (**HILYS**, $n = 19$). Both diets were isoenergetic. Jugular blood samples obtained on days 90 and 110 of gestation were used to measure concentrations of insulin-like growth factor-1 (**IGF-1**), metabolites, and amino acids (**AA**). Gilts were necropsied on day 110 ± 1 of gestation to obtain mammary glands for compositional analyses, immunohistochemistry, and analysis of mRNA abundance for AA transporters and markers of cell proliferation and differentiation. The HILYS gilts gained more body weight ($P < 0.01$) during the experimental period compared with CTL gilts, and had greater fetal weights (1.29 vs. 1.21 ± 0.03 kg, $P < 0.05$). There was no difference in circulating IGF-1, glucose, or albumin ($P > 0.10$) between HILYS and CTL gilts on day 110 of gestation, whereas concentrations of urea and free fatty acids were greater ($P < 0.01$), and those of Trp and Ala were lower ($P < 0.05$), in HILYS than CTL gilts. The provision of lysine at 40% above estimated requirements increased total mammary parenchymal mass by 44%, as well as total parenchymal fat, protein, DNA, and RNA ($P < 0.01$). The mRNA abundance of *ACACA* was greater ($P < 0.05$) in HILYS than CTL gilts, while only the AA transporter *SLC6A14* tended ($P < 0.10$) to be greater. Results demonstrate that providing dietary Lys above current National Research Council recommendations in late gestation increases mammary development in gilts. Results also indicate that Lys may have been limiting for protein retention. These data suggest that the use of a two-phase feeding strategy during gestation, whereby dietary Lys is increased from day 90, could benefit potential sow milk yield in the subsequent lactation.

Lay Summary

Results indicate that the current National Research Council recommendations for dietary lysine during late pregnancy in pigs, the period when most mammary gland development takes place, are underestimated. From days 90 to 110 of gestation, gilts were fed 2.65 kg of either a conventional diet providing 18.6 g/d of standardized ileal digestible (**SID**) lysine, or a diet providing 26.0 g/d of SID lysine via the inclusion of additional soybean meal. Diets were isoenergetic. Feeding 26.0 g/d of SID lysine increased the mass of mammary parenchymal tissue (where milk is synthesized) by 44%. Findings suggest that a greater mammary uptake of lysine in supplemented sows supported enhanced accretion of mammary parenchyma. Such information is most pertinent in the actual context where milk yield of hyperprolific sows must be maximized to sustain optimal growth of all their piglets. Furthermore, these data indicate that the use of a two-phase feeding strategy during gestation, whereby dietary lysine is increased from day 90, could benefit potential sow milk yield in the subsequent lactation.

Key words: feeding, gestation, gilt, lysine, mammary development, swine

Abbreviations: AA, amino acids; BF, backfat thickness; BW, body weight; CTL, control; FFA, free fatty acids; HILYS, high lysine; IGF-1, insulin-like growth factor-1; pSTAT5A, phosphorylated signal transducer and activator of transcription 5A; SID, standardized ileal digestible

Introduction

Lysine is the first limiting amino acid (AA) in most swine diets. Numerous studies were conducted to investigate its requirement prior to parturition (Gourley et al., 2020) and during lactation (NRC, 2012; Gourley et al., 2017; Hojgaard et al., 2019; Greiner et al., 2020) to support optimal sow performance. These studies are important given the current increase in litter size which has led to impedance of piglet growth due to inadequate milk supply (Kobek-Kjeldager et al., 2020). The role of dietary Lys supply for reproductive performance in gilts may start as early as during the growing period, where increasing the Lys-to-energy ratio in prepuberty and gestation improved the subsequent performance of their litters as reflected by increased piglet birth weight, number of piglets weaned, and average piglet weaning weight in first parity (Tuong et al., 2021). However, it is not known if this beneficial effect was due to the greater Lys intake before or after mating. Furthermore, another study showed no increase in piglet birth weight or average daily gain during lactation with semi ad libitum compared with restricted feeding for an 80 d period before mating (Klaaborg et al., 2019). During gestation, it was reported that an increase of total Lys intake above 11 g/d from day 5 (Thomas et al., 2021) or day 30 of gestation (Cooper

Received January 19, 2022 Accepted March 24, 2022.

et al., 2001) did not improve sow productivity, although the focus of these studies was on sow body weight (**BW**) changes without considering mammary tissue accretion.

Lysine is most important during late gestation when the majority of fetal and mammary growth occurs. More specifically, extensive accretion of mammary tissue takes place beyond day 90 of gestation (Sorensen et al., 2002), yet the specific AA requirements to support this essential process are not known. The energy requirement during late pregnancy in gilts and sows is relatively constant, whereas their protein requirements increase markedly (Ji et al., 2006; Krogh et al., 2020). The standardized ileal digestible (**SID**) Lys requirement for sows during gestation was reported to increase from 5.6 g/d in the first 60 d of gestation to 8.8 g/d for the remainder of gestation (Kim et al., 2009), and from 6.8 g/d in the first 70 d of gestation to 15.3 g/d thereafter for gilts (Ji et al., 2005). Samuel et al. (2012) also determined such an increase in Lys requirement between early (24 to 45 d) and late (86 to 110 d) gestation in sows. During the last 12 d of gestation, mammary growth was estimated to account for 16.8% of SID Lys requirements (Feyera and Theil, 2017). Early studies showed no effect of increasing protein (from 216 to 330 g/d; Weldon et al., 1991) or Lys intake (4, 8, or 16 g/d; Kusina et al., 1999a) on mammary development during late gestation. However, the 4 and 8 g/d treatments in the latter study failed to support maximal milk yield in the subsequent lactation (Kusina et al. 1999b). In a recent study, the provision of 20.6 g/d instead of 14.7 g/d of SID Lys to sows from day 90 of gestation increased piglet weight gain in the following lactation (Che et al., 2019). One possible explanation for this positive outcome was enhanced mammary development due to greater intake of AA.

When the uptake of nutrients by the mammary glands and their rates of extraction were used to estimate the supply of essential AA to the mammary glands, it appeared not to be a limiting factor for the growth of the mammary parenchyma in late pregnant sows (Krogh et al., 2017). The uptake of AA by mammary tissue depends on specific transport systems, where Lys is predominantly transported via the CAT-1 (encoded by *SLC7A1*) and $ATB^{0,+}$ (encoded by *SLC6A14*) transporters (reviewed by Wu et al., 2020). Expression of the genes for these transporters and those involved in cell proliferation and differentiation may be altered by supplementary Lys. Therefore, the goal of the present study was to determine the impact of a 40% increase in SID Lys intake from days 90 to 110 of gestation in gilts on mammary development, metabolic status, and mammary mRNA abundance of AA transporters and markers of cell proliferation and differentiation.

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

A total of 38 Yorkshire FAST × Landrace FAST gilts (Groupe Cérès Inc., Saint-Nicolas, QC, Canada) were bred via artificial insemination using pools of semen from Duroc Super Gain Plus boars (Centre d'Insémination Porcine du Québec, Saint-Lambert-de-Lauzon, QC, Canada). Gestating gilts were housed in individual pens (1.5 × 2.4 m), from mating until day 89 of gestation, they were fed one daily meal (0800 hours) of a conventional corn-based diet (12.75 MJ/kg DE, 11.24% CP, and 0.57% lysine). The amounts fed were determined from a commercial chart based on BW and backfat thickness (**BF**) as follows: gilts weighing 135 to 159 kg at mating were fed 2.45, 2.30, 2.15, and 1.95 kg, respectively, for a BF of ≤9, 10 to 12, 13 to 15, or ≥16 mm. Gilts weighing 160 to 194 kg at mating were fed 2.65, 2.50, 2.30, and 2.05 kg for the same BF categories. From day 90 of gestation, all gilts were fed 2.65 kg of either a conventional diet (**CTL**, control, $n = 19$) providing 18.6 g/d of SID Lys with all other AA meeting or exceeding National Research Council (NRC) recommendations (2012) or a diet providing 26.0 g/d of SID Lys via the inclusion of additional soybean meal (**HILYS**, $n = 19$) with all other AA to Lys ratios meeting or exceeding NRC recommendations. Diets were isoenergetic on a net energy (NE) basis (see Table 1 for the composition of experimental diets). Feed samples for AA analysis were collected every 2 wk. Gilts were weighed and their BF was measured ultrasonically (WED-3000, Schenzhen Well D Medical Electronics Co., Guangdong, China) at P2 of the last rib at mating and on days 90 and 110 of gestation. On days 90 and 110 of gestation, blood samples were collected by jugular venipuncture before the meal (between 0700 and 0800 hours) following a 16 h fast. Gilts were necropsied on day 110 ± 1 of gestation to obtain mammary glands for compositional analyses, immunohistochemistry, and measures of mRNA abundance. The uterus was removed and fetuses were counted and weighed, and the ovaries were weighed and the number of corpora lutea was counted.

Blood handling and assays

The concentrations of insulin-like growth factor-1 (**IGF-1**), glucose, free fatty acids (**FFA**), urea, albumin, 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 the following day, before the serum was harvested. Blood samples for IGF-1, FFA, albumin, and AA assays (30 mL) were collected in EDTA tubes (Becton Dickinson), put on ice, and centrifuged within 20 min for 12 min at 1,800 × *g* at 4 °C, from which plasma was immediately recovered. Finally, 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 was recovered immediately. Serum and plasma samples 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 previously detailed (Plante et al., 2011). The assay was validated using a pooled plasma sample from sows, as previously described (Plante et al., 2011). Sensitivity of the assay was 0.10 ng/mL, while the intra- and interassay coefficients of variability (**CVs**) were 5.17% and 4.20%, respectively. Glucose was measured by an enzymatic colorimetric method (Wako Chemicals, Richmond, VA). Assay validation was performed 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 1.67% and 2.69%, respectively. Urea was measured colorimetrically using an autoanalyzer (Auto-Analyser 3; Technicon Instruments Inc., Tarrytown, NY) according to the method of Huntington

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

Item	CTL[1]	HILYS
Ingredient composition, %		
Corn	55.45	42.76
Wheat	20.00	20.00
Soybean meal, dehulled, solvent extracted	20.50	32.00
Soybean oil	0.92	2.18
Di-calcium phosphate	1.40	1.22
Limestone	1.15	1.15
Sodium chloride	0.30	0.30
Vitamin and mineral premix[2]	0.20	0.20
DL-Met	0	0.07
L-Thr	0	0.04
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, %	16.56	21.07
SID Lys, %[5]	0.70	0.98
SID Arg, %	0.94	1.27
SID His, %	0.40	0.50
SID Ile, %	0.58	0.77
SID Leu, %	1.24	1.50
SID Met, %	0.24	0.36
SID Met + Cys, %	0.50	0.67
SID Phe, %	0.71	0.91
SID Phe + Thr, %	1.17	1.50
SID Thr, %	0.50	0.70
SID Trp, %	0.17	0.24
SID Val, %	0.65	0.84
Total Ca, %	0.87	0.87
STTD phosphorus, %	0.37	0.37
Analyzed nutrient contents		
Crude protein, %	15.36	21.44
Lys, %	0.80 (0.82)[5]	0.94(1.12)
Arg, %	0.86 (1.02)	1.10 (1.37)
His, %	0.42 (0.45)	0.52 (0.57)
Ile, %	0.60 (0.66)	0.73 (0.87)
Leu, %	1.39 (1.41)	1.61 (1.71)
Met, %	0.24 (0.28)	0.33 (0.40)
Met + Cys, %	0.51 (0.59)	0.68 (0.77)
Phe, %	0.82 (0.81)	1.00 (1.04)
Phe + Tyr, %	- (1.34)	- (1.72)
Thr, %	0.55 (0.61)	0.69 (0.82)
Trp, %	- (0.20)	- (0.26)
Val, %	0.66 (0.76)	0.79 (0.97)
Ca, %	0.58	0.60
Phosphorus, %	0.55	0.58
Crude fat, %	3.45	2.47

[1]CTL: control, Lys provided at estimated requirements for gilts 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 kg 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; thiamin, 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 AA contents are shown in parentheses.

(1984). Intra- and interassay CVs were 3.29% and 0.50%, respectively. Concentrations of FFA were measured by colorimetry (Wako Chemicals) having intra- and interassay CVs of 1.94% and 4.05%, respectively. The plasma albumin concentrations were analyzed according to the manufacturer's instructions (Bromocresol Green [BCG] Albumin Assay; Sigma-Aldrich, St. Louis, MO). The intra- and interassay CVs were 4.79% and 3.72%, 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 (AOAC, 2005; method 994.12) and were quantified via ion-exchange chromatography with post-column derivatization with ninhydrin according to Llames and Fontaine (1994).

Mammary gland measurements

At necropsy, mammary glands from one side of the udder (aiming for seven glands) were excised for measures of 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 and the mammary parenchymal tissue was dissected from surrounding adipose tissue (i.e., extraparenchymal tissue) at 4 °C. Parenchyma from all dissected and sliced glands was homogenized and a representative sample was 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 of parenchymal tissue was evaluated in all samples using a method based on fluorescence of a DNA stain (Labarca and Paigen, 1980). Dry matter (method 950.46; AOAC, 2005), protein (method 928.08; AOAC, 2005), and lipid content (method 991.36, AOAC, 2005) were also determined in the parenchyma. Both RNA and DNA contents were reported on a dry matter basis.

The contralateral row of mammary glands was used to measure the relative mRNA abundance of selected genes in the parenchyma. Samples from the fourth anterior glands were frozen in liquid nitrogen within 15 min after necropsy and were stored at –80 °C. Samples from the fifth gland were collected for histology and immunohistochemistry, fixed in 4% neutral buffered paraformaldehyde for 24 h at 4 °C, then washed twice with and stored in 70% ethanol prior to embedding in paraffin.

Histology and immunohistochemistry

To measure the circumference of alveolar lumens, paraffin-embedded samples were sectioned at 4 µm and stained with hematoxylin and eosin. Random images were captured

(QICAM Fast 1394; Q-Capture Pro 7) from all corners and the center of each section. The circumference of the apical surface for at least 10 alveolar lumenae per field (average of 56 lumenae per animal) was manually traced (Image-Pro Express 6.3) and length was determined relative to a stage micrometer. For immunohistochemistry, paraffin-embedded serial sections (4 µm) were stained for Ki67 as an indicator of cell proliferation, or for phosphorylated signal transducer and activator of transcription 5A (**pSTAT5A**) as an indicator of activated cellular signaling. Sections were rehydrated and pretreated with 0.3% Triton-X in PBS before steaming in citrate buffer (pH = 6; Antigen Retrieval Solution; Sigma-Aldrich) and blocking for endogenous biotin (Avidin/Biotin Block, Vector Laboratories, Inc., Burlingame, CA). Sections were blocked with 10% horse serum then incubated overnight at 4 °C with either a rabbit monoclonal anti-Ki67 antibody (RRID:AB_2341197; 1:50; Thermo Fisher Scientific, Waltham, MA) or a rabbit monoclonal anti-pSTAT5A antibody (RRID:AB_823649; 1:50; Cell Signaling Technology, Danvers, MA; Phospho-STAT5 (Tyr694)(C11C5) Rabbit mAb detects endogenous levels of STAT5a only when phosphorylated at Tyr694). Sections were rinsed in 0.05% PBS-Tween20 and incubated with a biotinylated donkey anti-rabbit antibody (1:200; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) followed by horseradish peroxidase-conjugated streptavidin (1:350; Jackson ImmunoResearch Laboratories, Inc.). Immunoreactivity was detected as a maroon precipitate (ImmPACT NovaRED, Vector Laboratories, Inc., Burlingame, CA) against a hematoxylin counterstain. Random images were captured using a QICAM Fast 1394 camera with Q-Capture Pro 7 software from all corners and the center of each section. Immuno-positive and -negative nuclei were distinguished using a blue-filtered saturation threshold. On average, 223 nuclei from each section were assessed manually (Image-Pro Express 6.3; range = 133 to 369) to determine the proportion of mammary epithelial cells that were positive for Ki67 or pSTAT5A.

Total RNA extraction, complementary DNA synthesis, and mRNA abundance of selected genes in mammary tissue

A total of 13 CTL and 13 HILYS gilts were randomly selected for qPCR analysis. Isolation of total RNA from mammary parenchyma was performed using the RNeasy Mini kit (Qiagen, Toronto, ON, Canada) followed by DNAse I digestion directly on the columns. The concentration and integrity of extracted RNA were determined using spectrophotometry (ND-1000, NanoDrop Technologies Inc., Wilmington, DE) and the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA), respectively. The synthesis of cDNA was achieved using Superscript IV Reverse Transcriptase (200 U/mL; Thermo Fisher Scientific) and oligo(dT) 20 primers (50 µM).

The relative mRNA abundance of selected genes in mammary parenchyma was determined using real-time PCR (qPCR) as described by Caron et al. (2020). The expression of 15 candidate genes was analyzed (Table 2) including for 2 major milk proteins (alpha lactalbumin [*LALBA*]; beta casein [*CSN2*]; Manjarin et al., 2011), 9 AA transporters (solute carrier family 1 member 1 [*SLC1A1*]; solute carrier family 3 member 1 [*SLC3A1*]; solute carrier family 3 member 2 [*SLC3A2*]; solute carrier family 6 member 14 [*SLC6A14*]; solute carrier family 7 member 1 [*SLC7A1*]; solute carrier family 7 member 2 [*SLC7A2*]; solute carrier family 7 member 6 [*SLC7A6*]; solute carrier family 7 member 7 [*SLC7A7*]; solute carrier family 7 member 9 [*SLC7A9*]; Manjarin et al., 2011; Huber et al., 2016), 2 markers of cell division/proliferation (cytoskeleton associated protein 2 [*CKAP2*]; claspin [*CLSPN*]; Trott et al., 2021), 1 gene involved in de novo fatty acid biosynthesis (acetyl-CoA carboxylase alpha [*ACACA*]; Lv et al., 2015), and 1 gene involved in mammary epithelial cell transcytosis of IgG during colostrogenesis (Fc fragment of IgG receptor and transporter [*FCGRT*]; Stark et al., 2013). The qPCR reactions and cycling conditions were as previously described (Farmer and Palin, 2021). Table 2 provides details on primer sequences used for each gene with their corresponding amplification efficiencies ($E = 10^{[-1/slope]}$). For each gene, standard curves were generated with serial dilutions of cDNA pools (Labrecque et al., 2009) to provide relative mRNA quantification using the standard curve method (Applied Biosystems, 1997). A standard curve (in duplicate) was included in each 96-well plate to account for experimental variation across plates. The expression of three reference genes (Table 2) was also determined (Apoptosis inhibitor 5 [*API5*], Mitochondrial ribosomal protein L39 [*MRPL39*], and VAMP-associated protein B and C [*VAPB*]). Using the NormFinder algorithm (Vandesompele et al. 2002) from Excel-Tools-Add-ins, the combination of *API5* + *VAPB* was identified as the best combination of reference genes to be used for mRNA abundance normalization and to obtain relative quantification values for each gene. Mean values from triplicates were used for statistical analyses.

Statistical analyses

The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analyses. 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 the main effects of treatment. An ANOVA with heterogeneous variances was used when necessary. The ANOVA for the weight of fetuses included litter size as a covariate. Repeated measures ANOVA with the factors treatment (the error term being gilt within treatment) and day of gestation (the residual error being the error term) and the treatment by day interaction were also carried out 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

Growth, ovarian, fetal, and blood variables of gilts

Mean ages at mating were 222.4 and 223.5 ± 1.9 d for CTL and HILYS gilts, respectively. The BW and BF of gilts are shown in Table 3. The HILYS gilts had greater BW gain ($P < 0.01$) during the experimental period (days 90 to 110 of gestation) compared with CTL gilts, whereas the loss in BF did not differ ($P > 0.10$). Values for BW or BF on days 90 or 110 did not differ between treatments ($P > 0.10$). The total number of corpora lutea was not affected by treatment ($P > 0.10$) with a similar mean value of 20.5 for both treatment groups. The combined weight of both ovaries tended to be greater (26.9 vs. 24.0 ± 1.1 g, $P < 0.10$) in HILYS vs. CTL gilts. Litter size for both treatments was similar (15.7 vs. 14.3 ± 0.85 for HILYS and CTL gilts, respectively, $P > 0.10$), while the weight of fetuses corrected for litter size was greater

Table 2. Primer sequences used for real-time PCR amplification of candidate genes[1]

Genes	Primer sequences (5′–3′)[2]	GenBank accession no.	Product size (bp)	Amplification efficiency (%)[3]
Gene				
ACACA	(F)CAGAGCTAGGCTAGGAGGAATA (R)ATCCAGGTTTGCAGGATCAG	NM_001114269	94	99.37
CKAP2	(F)CTATGTCTTCTGGGCAGTAAGTG (R)GGGTACTGAGATCAAGGTAGGA	XM_003130956	101	99.38
CLSPN	(F)GTGACCAGGACTCAGTGAAAG (R)GCCTTGGGAAGAGGCATAAA	XM_021095922	104	103.53
CSN2	(F)AAGCCTTTCAAGCAGTGAGGAA (R)TCTGGCGTTCATTCTCTGTTTG	NM_214434	101	98.98
FCGRT	(F)CCTGAATGGCGAGGAGTTTAT (R)GGAAGGTCTTCTCCTTGTTGAC	NM_214197	131	102.08
LALBA	(F)TCCTGGATGATGACCTTACT (R)TCTGAACAGAGTGCTTTATGG	NM_214360	101	103.09
SLC1A1	(F)GTTACCCGTTGGTGCTACTATC (R)TGCCCAAGTCCAAGTCATTC	NM_001164649	98	99.49
SLC3A1	(F)CCAAACCACACCAGTGATAAAC (R)GGGTACAGTCATGCCAGATATAA	NM_001123042	94	101.43
SLC3A2	(F)CCACCAAGGACCTGTTGTTA (R)GCCAGTGGCATTCAAATACTG	XM_003353809	101	99.20
SLC6A14	(F)GTGTTCGCTGGATTTGCTATTT (R)GTGTTCGCTGGATTTGCTATTT	NM_001348402	128	100.21
SLC7A1	(F)CCATGCCGCGAGTTATCTAT (R)GAGGCTAACGTGGCGATTAT	NM_001012613	106	97.01
SLC7A2	(F)GGATGAGGATGAGGATGAAGATAC (R)TCAGGTGTCTTTGGTGATGG	NM_001110420	101	99.87
SLC7A6	(F)CCCGAAGGCCACTCTTTATT (R)ATATCTCCTCTGGCCTCTACTC	XM_021094151	144	100.95
SLC7A7	(F)GTGAGGAGAACCCACAGATTAG (R)GGAGGAGAAGAAAGCCTTCAG	NM_001110421	111	98.09
SLC7A9	(F)GACCAGCCTGTTCGTCATAA (R)CGTAGAAGGGCGAAGAAACA	NM_001110171	83	103.31
Reference genes				
API5	(F)TTGCAGACAGTGAGTGGAAG (R)GTCAGGATCCGAGGGATTAAAG	XM_003122856	90	100.22
MRPL39	(F)TCAGAACCCTGAGAGAATAGTCAAAC (R)TGCTGATACTTCATACTGGAAACAAA	XM_003132745	112	100.49
VAPB	(F)TGGCGCTGGTGGTTTTG (R)CCTACAAGGCGATCTTCCCTATG	NM_001123213	60	99.77

[1]*ACACA*, acetyl-CoA carboxylase alpha; *API5*, apoptosis inhibitor 5; *CKAP2*, cytoskeleton-associated protein 2; *CLSPN*, claspin; *CSN2*, casein beta; *FCGRT*, Fc fragment of IgG receptor and transporter; *LALBA*, lactalbumin alpha; *MRPL39*, mitochondrial ribosomal protein L39; *SLC1A1*, solute carrier family 1 member 1; *SLC3A1*, solute carrier family 3 member 1; *SLC3A2*, solute carrier family 3 member 2; *SLC6A14*, solute carrier family 6 member 14; *SLC7A1*, solute carrier family 7 member 1; *SLC7A2*, solute carrier family 7 member 2; *SLC7A6*, solute carrier family 7 member 6; *SLC7A7*, solute carrier family 7 member 7; *SLC7A9*, solute carrier family 7 member 9; *VAPB*, VAMP-associated protein B and C.
[2]Foward (F) and reverse (R) primers.
[3]Amplification efficiency (E) was calculated with $E = 10^{[-1/slope]}$.

in HILYS gilts (1.29 vs. 1.21 ± 0.03 kg, $P < 0.05$). When values for fetal weight were not corrected for litter size, treatments tended to differ ($P < 0.10$; 1.30 and 1.22 ± 0.03 kg for HILYS and CTL gilts, respectively).

Circulating concentrations of IGF-1 and various metabolites are shown in Table 4. There were no differences in IGF-1, glucose, or albumin ($P > 0.10$), whereas the concentrations of urea were greater ($P < 0.01$) in HILYS than CTL gilts on day 110 of gestation. Concentrations of FFA were greater on both days 90 ($P < 0.01$) and 110 ($P < 0.05$) of gestation in HILYS compared with CTL gilts. Values for AA that were measured in plasma are shown in Table 5. For essential AA, concentrations of Trp were lower ($P < 0.05$) in HILYS than CTL gilts on day 110 of gestation, while the concentrations of Met and Phe also tended to be lower ($P < 0.10$) in HILYS gilts. Concentrations of the nonessential AA, Ala were lower ($P < 0.01$) in HILYS than CTL gilts on day 110 of gestation, and tended to be lower ($P < 0.10$) before the onset of treatment on day 90. Concentrations of Glu and Gly were lower ($P < 0.05$), while those of Pro and Tyr tended to be lower ($P < 0.10$) on day 110 of gestation for HILYS compared with CTL gilts. Concentrations of the essential AA His and Lys, and those of the nonessential AA Asp, Gly, and Ser were lower ($P < 0.05$) on day 110 than on day 90 of gestation. On the other hand, concentrations of Tyr were greater ($P < 0.01$) on day 110 than on day 90 of gestation.

Table 3. Body weight and backfat thickness of gilts fed a control diet (CTL, *n* = 19) or a lysine-supplemented diet (HILYS, *n* = 19) from days 90 to 110 of gestation

Variable measured	Treatment			
	CTL	HILYS	SEM[1]	*P* value
BW[2], kg				
Mating	157.3	157.3	1.2	0.99
Day 90 of gestation	201.8	202.0	2.3	0.95
Day 110 of gestation	226.0	230.0	2.4	0.18
Gain from days 90 to 110	24.2[a]	28.0[b]	0.7	<0.001
Backfat thickness[3], mm				
Mating	18.3	18.3	0.6	0.96
Day 90 of gestation	17.8	17.8	0.5	0.99
Day 110 of gestation	17.6	17.2	0.5	0.55
Loss from days 90 to 110	0.2	0.6	0.2	0.20

[1]Maximum value for the standard error of the mean (SEM).
[2]Treatment by day effect ($P < 0.01$).
[3]Day effect ($P < 0.01$).
[a,b]Means within a row with different superscripts differ ($P < 0.01$).

Table 4. Circulating concentrations of IGF-1, glucose, free fatty acids, urea, and albumin for gilts fed a control diet (CTL, *n* = 19) or a lysine-supplemented diet (HILYS, *n* = 19) from days 90 to 110 of gestation

Variable measured	Treatment			
	CTL	HILYS	SEM[1]	*P*-value
IGF-1[2], ng/mL				
Day 90 of gestation	39.5	35.5	3.1	0.38
Day 110 of gestation	45.0	41.1	3.2	0.39
Glucose, mMol/L				
Day 90 of gestation	3.36	3.25	0.10	0.47
Day 110 of gestation	3.32	3.35	0.08	0.79
FFA[3], µEq/L				
Day 90 of gestation	115.8[a]	185.3[b]	16.7	0.006
Day 110 of gestation	144.1[c]	258.0[d]	38.3	0.04
Urea[4], mMol/L				
Day 90 of gestation	3.78	3.77	0.21	0.96
Day 110 of gestation	6.44[a]	8.34[b]	0.24	<0.001
Albumin, g/dL				
Day 90 of gestation	5.08	4.87	0.12	0.23
Day 110 of gestation	5.13	5.01	0.12	0.48

[1]Maximum value for the standard error of the mean (SEM).
[2]Day effect ($P < 0.01$).
[3]Day effect ($P < 0.05$).
[4]Treatment by day effect ($P < 0.01$).
[a,b]Means within a row with different superscripts differ ($P < 0.01$).
[c,d]Means within a row with different superscripts differ ($P < 0.05$).

Mammary gland variables

Mammary gland composition and the abundance of mRNAs for candidate genes in the mammary parenchyma of gilts are shown in Tables 6 and 7, respectively. Feeding supplementary Lys increased mammary parenchymal mass ($P < 0.01$) by 44% and 42% for total and per teat values, respectively, but did not affect extraparenchymal tissue mass ($P > 0.10$). The only variable of parenchymal composition that changed was percent dry matter, which was greater ($P < 0.05$) in HILYS vs. CTL gilts, and there was also a tendency for an increased percent protein ($P < 0.10$) in HILYS gilts. On the other hand, the total amount of the parenchyma variables of fat, protein, DNA, DNA/teat, RNA, and RNA/teat were all increased ($P < 0.01$) in HILYS gilts. There was no treatment effect ($P > 0.10$) on alveolar circumference or on the abundance of mammary epithelial cell nuclei that were positive for either Ki67 or pSTAT5A (Table 6).

The abundance of mRNA for *ACACA* was greater ($P < 0.05$), and that for *SLC6A14* tended to be greater ($P < 0.10$), in the mammary parenchyma from HILYS

Table 5. Circulating concentrations of essential and nonessential AA in gilts fed a control diet (CTL, *n* = 19) or a lysine-supplemented diet (HILYS, *n* = 19) from days 90 to 110 of gestation

Variable measured	Treatment			
	CTL	HILYS	SEM[1]	*P*-value
Essential AA, µMol/L				
Arg[2]				
Day 90	50[e]	75[f]	9.3	0.06
Day 110	107	98	4.6	0.20
His[3]				
Day 90	85	83	2.0	0.48
Day 110	79	76	2.3	0.28
Ile				
Day 90	98	111	7.3	0.20
Day 110	115	113	4.8	0.74
sLeu				
Day 90	156	163	25.4	0.85
Day 110	133	126	4.5	0.24
Lys[3]				
Day 90	133	133	8.0	0.95
Day 110	96	98	5.6	0.77
Met[4]				
Day 90	45	46	1.8	0.89
Day 110	42[e]	35[f]	2.4	0.06
Phe				
Day 90	75	76	3.5	0.89
Day 110	74[e]	68[f]	2.0	0.06
Thr[4]				
Day 90	124	120	4.7	0.59
Day 110	107	114	3.4	0.11
Trp[5]				
Day 90	57	54	2.3	0.37
Day 110	60[c]	53[d]	2.1	0.02
Val				
Day 90	211	179	23.7	0.34
Day 110	187	194	5.1	0.31
Nonessential AA, µMol/L				
Ala[4]				
Day 90	446[e]	414[f]	13.1	0.09
Day 110	431[a]	362[b]	14.5	0.002
Asp[6]				
Day 90	9	8	0.5	0.58
Day 110	8	7	0.4	0.20
Cys				
Day 90	10	10	1.1	0.99
Day 110	9	8	0.8	0.20
Glu[4]				
Day 90	128	131	5.8	0.74
Day 110	119[c]	104[d]	4.6	0.03
Gly[3]				
Day 90	1012	991	48.7	0.76
Day 110	813[c]	711[d]	28.7	0.02
Pro				
Day 90	196	198	6.1	0.81
Day 110	197[e]	183[f]	5.0	0.06
Ser[3]				
Day 90	112	111	2.8	0.71
Day 110	104	101	3.1	0.43
Tyr[3,5]				
Day 90	89	84	3.6	0.42
Day 110	103[e]	93[f]	3.4	0.05

[1]Maximum value for the standard error of the mean (SEM).
[2]Treatment by day interaction ($P < 0.05$).
[3]Day effect ($P < 0.01$).
[4]Tendency for a treatment by day interaction ($P < 0.10$).
[5]Tendency for a treatment effect ($P < 0.10$).
[6]Day effect ($P < 0.05$).
[a,b]Means within a row with different superscripts differ ($P < 0.01$).
[c,d]Means within a row with different superscripts differ ($P < 0.05$).
[e,f]Means within a row with different superscripts tend to differ ($P < 0.10$).

compared with CTL gilts. On the other hand, mRNA abundance for *CKAP2* ($P < 0.05$), *CLSPN* ($P < 0.05$), and *SLC3A2* ($P < 0.01$) were lower in gilts fed supplementary Lys vs. in CTL gilts.

Discussion

Current results provide the first demonstration that an increase in dietary protein to supplement Lys above the NRC recommendations during late gestation (NRC, 2012) increases the accretion of mammary parenchymal tissue in gilts. The period of treatment from days 90 to 110 of gestation is when the mammary gland undergoes the majority of its development (Sorensen et al., 2002) and is responsive to external stimuli. Indeed, exogenous porcine somatotropin administered during the same period increased mammary development, with greater parenchymal mass and increased concentrations of both parenchymal DNA and RNA (Farmer and Langendijk, 2019). Interestingly, the present findings show a 44% increase in parenchymal tissue mass in response to greater Lys and protein intakes, compared with a 22% increase in response to exogenous porcine somatotropin (Farmer and Langendijk, 2019), although the mode of action likely differs given that parenchymal DNA and RNA concentrations were unchanged in the current study. Furthermore, there was no evidence of precocious milk secretion or accumulation as indicated by the similar diameter of mammary alveoli. It is therefore likely that there were more parenchymal cells of similar size amassed in HILYS gilts. Even though there was no change in the percentage of Ki67 positive cells at necropsy at day 110, enhanced cell division may have occurred prior to this time. Indeed, the proliferation of mammary epithelial cells was greater on day 90 than on day 110 of gestation in primiparous gilts (VanKlompenberg et al., 2013). In accordance with this proposal, plasma Lys concentrations were positively related to mammary cell division, vs. differentiation, in female offspring 24 h after birth (Bitsie et al., 2021). Furthermore, the drastic increase in total parenchymal tissue of HILYS gilts indicates that increased cell division must have occurred at some time. Hence the lower expression level for *CKAP2*, being associated with hyperplasia, was surprising and further suggests that enhanced division likely occurred earlier and was no longer apparent on day 110 of gestation.

Table 6. Mammary gland composition and immunohistochemical variables for parenchymal tissue from gilts fed a control diet (CTL, *n* = 19) or a lysine-supplemented diet (HILYS, *n* = 19) from days 90 to 110 of gestation

Variable measured	Treatment			
	CTL	HILYS	SEM[1]	*P* value
Extraparenchymal tissue, g	1,691.0	1,690.2	64.8	0.99
Parenchymal tissue, g	1,437.4[a]	2,073.6[b]	121.9	<0.001
Parenchyma/teat, g	189.0[a]	268.5[b]	16.2	0.001
Parenchymal tissue composition				
Dry matter, %	37.8[c]	38.8[d]	0.7	0.04
Fat, % of dry matter	63.3	60.3	1.5	0.17
Fat, g total	337.2[a]	437.9[b]	21.3	0.002
Protein, % of dry matter	32.9[e]	35.9[f]	1.2	0.09
Protein, g total	178.4[a]	267.9[b]	18.8	0.002
DNA, mg/g on dry matter basis	6.59	7.06	0.28	0.24
DNA, g total	3.58[a]	5.25[b]	0.36	0.002
DNA/teat, g	0.47[a]	0.68[b]	0.05	0.003
RNA, mg/g on dry matter basis	7.76	8.38	0.29	0.14
RNA, g total	4.20[a]	6.15[b]	0.37	<0.001
RNA/teat, g	0.55[a]	0.80[b]	0.05	0.001
Alveolar circumference, µm	141	151	10	0.35
Ki67positive epithelial cells, %	8.5	11.3	2.4	0.33
pSTAT5A positive epithelial cells, %	43.2	48.2	4.3	0.38

[1]Maximum value for the standard error of the mean (SEM).
[a,b]Means within a row with different superscripts differ ($P < 0.01$).
[c,d]Means within a row with different superscripts differ ($P < 0.05$).
[e,f]Means within a row with different superscripts tend to differ ($P < 0.10$).

A previous report indicated that mammary development in gilts on day 105 was unchanged when dietary protein was increased from 216 to 330 g of crude protein per day from day 75 of gestation (Weldon et al., 1991). On the other hand, when Kusina et al. (1999a, 1999b) fed 4, 8, or 16 g/d of Lys to gilts from day 25 of gestation, the two lower doses failed to support maximal milk yield in the subsequent lactation (Kusina et al., 1999b), even though mammary development on day 108 of gestation was unchanged (Kusina et al., 1999a). This beneficial effect of the highest Lys intake was proposed as being due to increased deposition of lean body mass in treated gilts. In the present study, HILYS gilts had a greater growth rate than CTL gilts, whereas BW was unchanged at the end of gestation, although whole-body and maternal chemical composition were not measured. It is important to mention that the daily intakes of SID Lys in the present study were 18.6 and 26.0 g/d for CTL and HILYS gilts, respectively, raising the possibility that the amounts of LYS fed by Kusina et al. (1999a) may have been insufficient to promote additional mammary development. The increase in mammary development reported herein also complements the findings from other recent studies, whereby increasing Lys and protein intakes in late gestation (Che et al., 2019) or during late gestation and lactation (Heo et al., 2008; Yang et al., 2008) improved the growth rate of suckling piglets. The greater weight of fetuses from HILYS gilts in the current study aligns with the 100 g increase (the tendency for a treatment effect) in birth weight reported by Che et al. (2019), and would also contribute to improved BW gain of piglets during the lactation period. On the other hand, Gonçalves et al (2016) reported no effect of feeding 20.0 g/d instead of 10.7 g/d of SID Lys from day 90 of gestation on piglet birth weight.

The mammary glands increase in weight by 50% when nursed during lactation, and an extra 0.96 g/d of total dietary Lys is needed for each piglet over 6 in a litter to support mammary growth (Kim et al., 1999). Even though mammary development was taken into consideration when nutrient requirements were estimated (NRC, 2012), the drastic increase in mammary parenchyma occurring in late gestation is most likely greater than expected. A previous report indicated that pregnant gilts require 15.3 g/d of SID Lys after day 70 of gestation (Ji et al., 2005), yet present results demonstrate that this was an underestimation. Che et al. (2019) also reported Lys requirements of at least 20.6 g/d for gilts from day 90 of gestation, while Zhang et al. (2011) suggested that Lys levels should be 0.65% to 0.75% of the diet in mid to late gestation, which is greater than current NRC recommendations (NRC, 2012). The criteria used to estimate Lys requirement, such as nitrogen retention, sow BW change, or piglet growth, affects the values obtained (Dourmad and Étienne, 2002) and it is only recently that mammary development has been considered in such estimations. Indeed, Samuel et al. (2012) noted that Lys requirements for gestating sows are less than current recommendations during early gestation (days 24 to 45), but are greater than current recommendations during late gestation (days 86 to 110), further highlighting that the dietary content of Lys needs to be increased in late pregnancy. There is no doubt that the true biological needs for Lys in gilts vary throughout pregnancy, and that feeding management should be adjusted to reflect this reality and to support optimal milk yield. Current results

Table 7. Abundance of mRNA for candidate genes in mammary parenchyma of gilts fed a control diet (CTL, *n* = 13) or a lysine-supplemented diet (HILYS, *n* = 13) from days 90 to 110 of gestation[1,2]

	Treatment			
Genes	CTL	HILYS	SEM[3]	*P*-value
ACACA	0.80[c]	1.16[d]	0.16	0.04
CKAP2	1.04[c]	0.84[d]	0.06	0.03
CLSPN	1.13[c]	0.95[d]	0.07	0.04
CSN2	0.93	1.22	0.21	0.21
FCGRT	1.03	0.94	0.05	0.20
LALBA	0.78	1.60	0.58	0.19
SLC1A1	1.05	1.02	0.15	0.89
SLC3A1	0.92	1.00	0.12	0.58
SLC3A2	1.14[a]	0.79[b]	0.04	<0.001
SLC6A14	0.70[e]	1.67[f]	0.50	0.07
SLC7A1	0.95	0.99	0.05	0.49
SLC7A2	0.89	0.92	0.07	0.74
SLC7A6	0.98	1.06	0.06	0.26
SLC7A7	1.11	0.98	0.11	0.36
SLC7A9	0.91	1.19	0.15	0.15

[1]*ACACA*, acetyl-CoA carboxylase alpha; *CKAP2*, cytoskeleton-associated protein 2; *CLSPN*, claspin; *CSN2*, casein beta; *FCGRT*, Fc fragment of IgG receptor and transporter; *LALBA*, lactalbumin alpha; *SLC1A1*, solute carrier family 1 member 1; *SLC3A1*, solute carrier family 3 member 1; *SLC3A2*, solute carrier family 3 member 2; *SLC6A14*, solute carrier family 6 member 14; *SLC7A1*, solute carrier family 7 member 1; *SLC7A2*, solute carrier family 7 member 2; *SLC7A6*, solute carrier family 7 member 6; *SLC7A7*, solute carrier family 7 member 7; *SLC7A9*, solute carrier family 7 member 9.
[2]Values correspond to relative mRNA abundance as determined with the standard curve method, described in materials and methods.
[3]Maximum value for the standard error of the mean (SEM).
[a,b]Means within a row with different superscripts differ ($P < 0.01$).
[c,d]Means within a row with different superscripts differ ($P < 0.05$).
[e,f]Means within a row with different superscripts tend to differ ($P < 0.10$).

provide critical information to substantiate this concept. The use of a two-phase feeding program for gestating swine was already proposed by others (Ji et al., 2005; Kim et al., 2009; Gaillard et al., 2020), and would definitely be warranted for optimal mammary development.

The similar circulating Lys concentrations across treatments suggest there was greater uptake of Lys by the mammary gland, and possibly other tissues, in HILYS sows to support the enhanced deposition of parenchymal tissue. Among the circulating AA, Lys is taken up by the mammary glands of lactating sows with the greatest efficiency (Trottier et al., 1997). Furthermore, the decreased concentrations of several circulating AA in HILYS gilts in the present study suggest that Lys was limiting protein synthesis in CTL gilts. The greater supply of dietary Lys would then have also led to other AA being taken up from the circulation. This hypothesis is opposite to the findings of Che et al. (2019), whereby concentrations of valine, isoleucine, leucine, and arginine in the circulation of lactating sows supplemented with Lys were increased 1 h after the onset of farrowing. If the ratio of various AA to Lys differed between the experiments, it could have affected their uptake by mammary tissue. However, considering the similar feed ingredients used in both studies, this would not be expected. Zhuo et al. (2020) also noted increased concentrations of various AA on the day of farrowing when sows were fed supplementary soybean bioactive peptides (containing mainly arginine and glutamine as functional AA) from day 90 of gestation.

The uptake of Lys into the mammary glands of sows occurs primarily via four cationic acid transporter systems, where both Lys and Arg have the same critical transporter systems, namely CAT-1 and $ATB^{0,+}$ that are encoded by the *SLC7A1* and *SLC6A14* genes, respectively (reviewed by Wu et al., 2020). There was only a tendency for an increase in parenchymal gene expression for *SLC6A14* and a decreased gene expression for *SLC3A2* in response to HILYS. Expression of the milk protein genes *CSN2* and *LALBA* was also unaffected by treatment. The lack of any pronounced change in response to HILYS could be explained by the fact that mRNA abundance for both the CAT-1 and $ATB^{0,+}$ AA transporters and for milk proteins is lower prior to farrowing compared with early or late lactation (Manjarin et al., 2011). Others previously reported a positive effect of supplemental Lys on mRNA abundance for *SLC6A14* in bovine mammary epithelial cells (Lin et al., 2018), whereas Huber et al. (2016) found no effect of dietary AA balance on mRNA abundance in the mammary glands for genes encoding Lys transporters in lactating sows. On the other hand, the increased expression of *ACACA* in HILYS gilts in the present study is generally associated with enhanced cellular differentiation, yet its expression level is very low in gestation (VanKlompenberg et al., 2013).

The increased circulating concentrations of urea in HILYS gilts at the end of the treatment period likely reflect the greater dietary protein or more use of protein as an energy source, as was the case in the studies by Che et al. (2019) and Hong et al. (2020). The greater concentrations of FFA in plasma at the end of gestation in HILYS gilts may reflect an enhanced need for energy to support increased protein synthesis and the elimination of excess AA. On the other hand, FFA concentrations were already greater in HILYS than CTL gilts at the onset of treatment, but the increase over the experimental period was greater in HILYS than CTL gilts. In keeping with the findings of Che et al. (2019), no increases in circulating glucose or albumin at the end of gestation were recorded in the current study.

In conclusion, these findings demonstrate that the previously reported increase in the growth rate of suckling piglets raised by sows fed diets with greater Lys from day 90 of gestation (Che et al., 2019) is likely attributable to enhanced development of the mammary parenchyma during this critical period. These data indicate that current NRC recommendations (NRC, 2012) for Lys during the late pregnancy phase of rapid mammary accretion are underestimated. However, it cannot be discounted that the greater concentrations of other AA may have also come into play. Such information is most pertinent in the actual context where milk yield of hyperprolific sows must be maximized to sustain the optimal growth of all their piglets.

Acknowledgments

We wish to thank A. Bernier, G. Bernatchez, É. Belley, and J. Zhu for their invaluable technical assistance, the staff of the Swine Complex, especially B. Paquette and D. Morissette, for care and slaughter of the animals, and S. Méthot for statistical analyses. Thanks to Swine Innovation Porc, CEVA Santé Animale and Zinpro Corp. for funding.

Conflict of interest statement

All authors declare that they have no conflict of interest.

LITERATURE CITED

AOAC. 2005. *Official methods of analysis international*. 18th ed. Arlington (VA): Association of Official Analytical Chemists.

Applied Biosystems. 1997. *ABI PRISM 7700 sequence detections system. User bulletin No. 2*. Foster City (CA): Applied Biosystems.

Bitsie, B., E. K. Ison, L. P. Jenkins, R. Klopp, C. McCabe, K. Mills, G. Nicholls, A. Richards, L. Shirley, K. Teeple, et al. 2021. Mammary development in gilts at one week postnatal is related to plasma lysine concentration at 24 h after birth, but not colostrum dose. *Animals*. 11:1–19. doi:10.3390/ani11102867

Boogers, I., W. Plugge, Y. Q. Stokkermans, and A. L. L. Duchateau. 2008. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatization method. *J. Chromatogr. A*. 1189: 406–409. doi:10.1016/j.chroma.2007.11.052

Caron, A., M. F. Palin, R. C. Hovey, J. Cohen, J. P. Laforest, and C. Farmer. 2020. Effects of sustained hyperprolactinemia in late gestation on mammary development of gilts. *Domest. Anim. Endocrinol*. 72:106408. doi:10.1016/j.domaniend.2019.106408

CCAC. 2009. *Canadian council on animal care guidelines on the care and use of farm animals in research, teaching and testing*. Ottawa (ON): Canadian Council on Animal Care in Science.

Che, L., L. Hu, C. Wu, Q. Xu, Q. Zhou, X. Peng, Z. Fang, Y. Lin, S. Xu, B. Feng, et al. 2019. Effects of increased energy and amino acid intake in late gestation on reproductive performance, milk composition, metabolic, and redox status of sows. *J. Anim. Sci*. 97: 2914–2926. doi:10.1093/jas/skz149

Cooper, D. R., J. F. Patience, R. T. Zijlstra, and M. Rademacher. 2001. Effect of energy and lysine intake in gestation on sow performance. *J. Anim. Sci*. 79:2367–2377. doi:10.2527/2001.7992367x

Dourmad, J. Y., and M. Etienne. 2002. Dietary lysine and threonine requirements of the pregnant sow estimated by nitrogen balance. *J. Anim. Sci*. 80:2144–2150. doi:10.2527/2002.8082144x

Farmer, C., and P. Langendijk. 2019. Exogenous porcine somatotropin stimulates mammary development in late-pregnant gilts. *J. Anim. Sci*. 97:2433–2440. doi:10.1093/jas/skz136

Farmer, C., and M. F. Palin. 2021. Hyperprolactinemia using domperidone in prepubertal gilts: effects on hormonal status, mammary development and mammary and pituitary gene expression. *Domest. Anim. Endocrinol*. 76:1–12. doi:10.1016/j.domaniend.2021.106630

Feyera, T., and P. K. Theil. 2017. Energy and lysine requirements and balances of sows during transition and lactation: a factorial approach. *Livest. Sci*. 201:50–57. doi:10.1016/j.livsci.2017.05.001

Gaillard, C., N. Quiniou, R. Gauthier, L. Cloutier, and J. Y. Dourmad. 2020. Evaluation of a decision support system for precision feeding of gestating sows. *J. Anim. Sci*. 98:1–12. doi:10.1093/jas/skaa255

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

Gourley, K. M., G. E. Nichols, J. A. Sonderman, Z. T. Spencer, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, S. J. Kitt, et al. 2017. Determining the impact of increasing standardized ileal digestible lysine for primiparous and multiparous sows during lactation. *Transl. Anim. Sci*. 1:426–436. doi:10.2527/tas2017.0043

Gourley, K. M., A. J. Swanson, J. M. DeRouchey, M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. C. Woodworth. 2020. Effects of increased lysine and energy feeding duration prior to parturition on sow and litter performance, piglet survival, and colostrum quality. *J. Anim. Sci*. 98:1–10. doi:10.1093/jas/skaa105

Greiner, L., P. Srichana, J. L. Usry, C. Neill, G. L. Allee, J. Connor, K. J. Touchette, and C. D. Knight. 2020. Lysine (protein) requirements of lactating sows. *Transl. Anim. Sci*. 4:751–763. doi:10.1093/tas/txaa072

Heo, S., Y. X. Yang, Z. Jin, M. S. Park, B. K. Yang, and B. J. Chae. 2008. Effects of dietary energy and lysine intake during late gestation and lactation on blood metabolites, hormones, milk composition and reproductive performance in primiparous sows. *Can. J. Anim. Sci*. 88:247–255. doi:10.4141/CJAS07060

Hojgaard, C. K., T. S. Bruun, and P. K. Theil. 2019. Optimal lysine in diets for high-yielding lactating sows. *J. Anim. Sci*. 10:4268–4281. doi:10.1093/jas/skz286

Hong, J., L. H. Fang, and Y. Y. Kim. 2020. Effects of dietary energy and lysine levels on physiological responses, reproductive performance, blood profiles, and milk composition in primiparous sows. *J. Anim. Sci. Technol*. 62:334–347. doi:10.5187/jast.2020.62.3.334

Huber, L., C. F. de Lange, C. W. Ernst, U. Krogh, and N. L. Trottier. 2016. Impact of improving dietary amino acid balance for lactating sows on efficiency of dietary amino acid utilization and transcript abundance of genes encoding lysine transporters in mammary tissue. *J. Anim. Sci*. 94:4654–4665. doi:10.2527/jas.2016-0697

Huntington, G. B. 1984. Net absorption of glucose and nitrogenous compounds by lactating Holstein cows. *J. Dairy Sci*. 67:1919–1927. doi:10.3168/jds.S0022-0302(84)81525-8

Ji, F., W. L. Hurley, and S. W. Kim. 2006. Characterization of mammary gland development in pregnant gilts. *J. Anim. Sci*. 84:579–587. doi:10.2527/2006.843579x

Ji, F., G. Wu, J. R. Blanton, Jr, and S. W. Kim. 2005. Changes in weight and composition in various tissues of pregnant gilts and their nutritional implications. *J. Anim. Sci*. 83:366–375. doi:10.2527/2005.832366x

Kim, S. W., W. L. Hurley, G. Wu, and F. Ji. 2009. Ideal amino acid balance for sows during gestation and lactation. *J. Anim. Sci*. 87(E. Suppl.): E123–E132. doi:10.2527/jas.2008-1452

Kim, S. W., I. Osaka, W. L. Hurley, and R. A. Easter. 1999. Mammary gland growth as influenced by litter size in lactating sows: impact on lysine requirement. *J. Anim. Sci*. 77:3316–3321. doi:10.2527/1999.77123316x

Klaaborg, J., T. N. Carl, T. S. Bruun, A. V. Strathe, J. K. Bache, A. R. Kristensen, and C. Amdi. 2019. The effect of feeding strategy during rearing in a commercial setting on gilt body condition, lactation performance and culling rate in modern sows nursing large litters. *Livest. Sci*. 228:144–150. doi:10.1016/j.livsci.2019.08.011

Kobek-Kjeldager, C., V. A. Moustsen, P. K. Theil., and L. J. Pedersen. 2020. Effect of litter size, milk replacer and housing on behaviour and welfare related to sibling competition in litters from hyper-prolific sows. *Appl. Anim. Behav. Sci*. 230:1–9. doi:10.1016/j.applanim.2020.105046

Krogh, U., N. Oksbjerg, A. C. Storm, T. Feyera, and P. K. Theil. 2017. Mammary nutrient uptake in multiparous sows fed supplementary arginine during gestation and lactation. *J. Anim. Sci*. 95:2517–2532. doi:10.2527/jas.2016.1291

Krogh, U., S. van Vliet, T. Sonderby Bruun, T. Feyera, T. Hinrichsen, T. Friis Pedersen, and P. Theil. 2020. Impact of dietary energy to protein ratio and two different energy levels during late gestation on plasma metabolites and colostrum production in sows. *Livest. Sci*. 234:1–8. doi:10.1016/j.livsci.2020.103999

Kusina, J., J. E. Pettigrew, A. F. Sower, M. R. Hathaway, M. E. White, and B. A. Crooker. 1999a. Effect of protein intake during gestation on mammary development of primiparous sows. *J. Anim. Sci*. 77:925–930. doi:10.2527/1999.774925x

Kusina, J., J. E. Pettigrew, A. F. Sower, M. E. White, B. A. Crooker, and M. R. Hathaway. 1999b. Effect of protein intake during gestation and lactation on the lactational performance of primiparous sows. *J. Anim. Sci*. 77:931–941. doi:10.2527/1999.774931x

Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem*. 102:344–352. doi:10.1016/0003-2697(80)90165-7

Labrecque, B., D. Beaudry, M. Mayhue, C. Hallé, V. Bordignon, B. D. Murphy, and M. F. Palin. 2009. Molecular characterization and expression analysis of the porcine paraoxonase 3 (PON3) gene. *Gene* 443:110–120. doi:10.1016/j.gene.2009.04.026

Lin, X., S. Li, Y. Zou, F. Q. Zhao, J. Liu, and H. Liu. 2018. Lysine stimulates protein synthesis by promoting the expression of ATB0,+ and activating the mTOR pathway in bovine mammary epithelial cells. *J. Nutr*. 148:1426–1433. doi:10.1093/jn/nxy140

Llames, C. R., and J. Fontaine. 1994. Determination of amino acids in feeds: collaborative study. *J. AOAC Int*. 77: 1362–1402.

Lv, Y., W. Guan, H. Qiao, C. Wang, F. Chen, Y. Zhang, and Z. Liao. 2015. Veterinary medicine and omics (Veterinomics): metabolic transition of milk triacylglycerol synthesis in sows from late pregnancy to lactation. *Omics* 19:602–616. doi:10.1089/omi.2015.0102

Manjarin, R., J. P. Steibel, V. Zamora, N. Am-in, R. N. Kirkwood, C. W. Ernst, P. S. Weber, N. P. Taylor, and N. L. Trottier. 2011. Transcript abundance of amino acid transporters, beta-casein, and alpha-lactalbumin in mammary tissue of periparturient, lactating, and postweaned sows. *J. Dairy Sci*. 94: 3467–3476. doi:10.3168/jds.2011-4163

NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed. Washington (DC): National Academic Press.

Plante, P.-A., J.-P. Laforest, and C. Farmer. 2011. Effect of supplementing the diet of lactating sows with NuPro® on their performances and that of their piglets. *Can. J. Anim. Sci*. 91: 295–300. doi:10.4141/cjas2010-008

Samuel, R. S., S. Moehn, P. B. Pencharz, and R. O. Ball. 2012. Dietary lysine requirement of sows increases in late gestation. *J. Anim. Sci*. 90:4896–4904. doi:10.2527/jas.2011-4583

Sorensen, M. T., K. Sejrsen, and S. Purup. 2002. Mammary gland development in gilts. *Livest. Prod. Sci*. 75: 143–148. doi:10.1016/S0301-6226(01)00310-4

Stark, A., E. Vachkova, O. Wellnitz, R. Bruckmaier, and C. Baumrucker. 2013. Colostrogenesis: candidate genes for IgG1 transcytosis mechanisms in primary bovine mammary epithelial cells. *J. Anim. Physiol. Anim. Nutr. (Berl)*. 97:1114–1124. doi:10.1111/jpn.12021

Thomas, L. L., L. K. Herd, R. D. Goodband, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. A. D. Goncalves, and D. B. Jones. 2021. Effects of increasing standardized ileal digestible lysine during gestation on reproductive performance of gilts and sows. *Animal* 15:100221. doi:10.1016/j.animal.2021.100221

Trott, J. F., A. Schennink, K. C. Horigan, D. G. Lemay, J. R. Cohen, T. R. Famula, J. A. Dragon, and R. C. Hovey. 2021. Unique transcriptomic changes underlie hormonal interactions during mammary histomorphogenesis in female pigs. *Endocrinology*. 163:1–27. doi:10.1210/endocr/bqab256

Trottier, N. L., C. F. Shipley, and R. A. Easter. 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. *J. Anim. Sci*. 75:1266–1278. doi:10.2527/1997.7551266x

Tuong, N. D., T. T. B. Ngoc, T. Hiep, and P. K. Dang. 2021. Effect of digestible lysine/metabolisable energy ratio in F1 (Landrace x Yorkshire) gilt diets on growth, age at puberty and reproductive performance in closed housing condition. *Adv. Anim. Vet. Sci*. 9: 1347–1354. doi:10.17582/journal.aavs/2021/9.9.1347.1354

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 3:RESEARCH0034. doi:10.1186/gb-2002-3-7-research0034

Vanklompenberg, M. K., R. Manjarin, J. F. Trott, H. F. McMicking, and R. C. Hovey. 2013. Late gestational hyperprolactinemia accelerates mammary epithelial cell differentiation that leads to increased milk yield. *J. Anim. Sci*. 91:1102–1111. doi:10.2527/jas.2012-5903

Volkin, E., and W. E. Cohn. 1954. Estimation of nucleic acids. *Methods Biochem. Anal*. 1:287–305. doi:10.1002/9780470110171.ch11

Weldon, W. C., A. J. Thulin, O. A. MacDougald, L. J. Johnston, E. R. Miller, and H. A. Tucker. 1991. Effects of increased dietary energy and mammary development in gilts. *J. Anim. Sci*. 69: 194–200. doi:10.2527/1991.691194x

Wu, Z., J. Heng, M. Tian, H. Song, F. Chen, W. Guan, and S. Zhang. 2020. Amino acid transportation, sensing and signal transduction in the mammary gland: key molecular signalling pathways in the regulation of milk synthesis. *Nutr. Res. Rev*. 33:287–297. doi:10.1017/S0954422420000074

Yang, Y., S. Heo, Z. Jin, J. Yun, P. Shinde, J. Choi, B. Yang, and B. Chae. 2008. Effects of dietary energy and lysine intake during late gestation and lactation on blood metabolites, hormones, milk composition and reproductive performance in multiparous sows. *Arch. Anim. Nutr*. 62:10–21. doi:10.1080/17450390701780227

Zhang, R. F., Q. Hu, P. F. Li, L. F. Xue, X. S. Piao, and D. F. Li. 2011. Effects of lysine intake during middle to late gestation (day 30 to 110) on reproductive performance, colostrum composition, blood metabolites and hormones of multiparous sows. *Asian-Aust. J. Anim. Sci*. 8: 1142–1147. doi:10.5713/ajas.2011.10449

Zhuo, Y., M. Cao, Y. Li, L. Tang, W. Li, X. Jiang, W. Xiao, S. Liu, X. Jiang, Z. Fang, et al. 2020. Soybean bioactive peptides supplementation during late gestation and lactation affect the reproductive performance, free amino acid composition in plasma and milk of sows. *Livest. Sci*. 237:1–8. doi:10.1016/j.livsci.2020.104064