Effects of dietary lysine intake during lactation on blood metabolites, hormones, and reproductive performance in primiparous sows

H. Yang, J. E. Pettigrew, L. J. Johnston, G. C. Shurson, J. E. Wheaton, M. E. White, Y. Koketsu, A. F. Sower and J. A. Rathmacher

J Anim Sci 2000. 78:1001-1009.

Effects of dietary lysine intake during lactation on blood metabolites, hormones, and reproductive performance in primiparous sows[1,2]

H. Yang*[,3], J. E. Pettigrew†, L. J. Johnston*, G. C. Shurson*, J. E. Wheaton*, M. E. White*, Y. Koketsu‡, A. F. Sower*, and J. A. Rathmacher§

*Department of Animal Science, University of Minnesota, St. Paul; †Pettigrew Consulting International, Louisiana, MO; ‡Department of Clinical and Population Science, University of Minnesota, St. Paul; and §Department of Animal Science, Iowa State University, Ames

ABSTRACT: Effects of three dietary lysine (protein) concentrations during lactation on metabolic state, protein metabolism, reproductive hormones, and performance were investigated in 36 primiparous sows. Sows were assigned randomly to one of three diets containing .4% (low lysine, LL), 1.0% (medium lysine, ML), or 1.6% (high lysine, HL) total lysine from intact protein sources. All diets contained 2.1 Mcal NE/kg and exceeded the recommended requirements for all other nutrients. Actual lysine intakes over an 18-d lactation were 16, 36, and 56 g/d for sows fed LL, ML, and HL, respectively. Fractional breakdown rate of muscle was determined on d 4 and 15 of lactation by using a three-compartment kinetic model of 3-methylhistidine metabolism. Increasing lysine intake during lactation did not affect fractional breakdown rate of muscle on d 4 of lactation but decreased it on d 15 ($P < .05$). Sows fed LL had a reduced number of LH pulses on d 12 and 18 ($P < .05$) and reduced serum estradiol (E_2) concentration on d 18 of lactation compared with sows fed ML and HL treatments. However, LH pulses and E_2 concentrations were similar between ML and HL treatments ($P > .35$). Increasing lysine intake increased serum urea nitrogen (SUN) and postprandial insulin concentrations ($P < .05$) during lactation but had no effect on plasma glucose concentrations ($P > .20$). Sows fed HL had greater serum IGF-I on d 6 and 18 than sows fed ML ($P < .05$). Number of LH peaks was correlated with serum insulin concentration 25 min after feeding on d 6 and 18 (r = .31 to .41; $P < .1$) and pre- (r = .33 to .46) and postprandial (r = .30 to .58) SUN concentrations ($P < .05$) during different stages of lactation. Results indicate that, compared with medium lysine intake, low lysine intake increased muscle protein degradation and decreased concentrations of insulin, SUN, and estradiol and LH pulsatility. In contrast, high lysine (protein) intake increased SUN, insulin, and IGF-I, but did not increase secretion of estradiol and LH compared with medium lysine intake. Furthermore, nutritional impacts on reproduction may be mediated in part through associated effects on circulating insulin concentration.

Key Words: Glucose, Insulin, Lactation, LH, Lysine, Sows

 J. Anim. Sci. 2000. 78:1001–1009

Introduction

Effects of nutrient intake during lactation on LH release and weaning-to-estrus interval may be mediated through changes in the metabolic state of the animal. The metabolic state can be partially assessed by measurement of circulating metabolites and metabolic hormones (Pettigrew and Tokach, 1993; Quesnel and Prunier, 1995). Insulin, IGF-I, and glucose are three of the most likely signals between nutritional status during lactation and subsequent reproductive performance (Pettigrew and Tokach, 1993; Booth et al., 1994; Koketsu et al., 1996).

A number of studies have demonstrated that low dietary lysine intake during lactation affects metabolic state, LH secretion, and weaning-to-estrus interval (King and Martin, 1989; Tokach et al., 1992; Jones and Stahly, 1999a) and increases mobilization of body protein (Jones and Stahly, 1999b). These studies focused on the influence of adequate to very low lysine intake during lactation but did not provide data on the responses to very high lysine intake during lactation. Increasing dietary lysine concentration above that re-

[1]Appreciation is expressed to Heartland Lysine, Inc., Chicago, IL; Pig Improvement Company, Franklin, KY; and Cargill, Inc., Minneapolis, MN, for financial support of this research.

[2]Published as paper no. 991020005 of scientific journal article series of the Minnesota Agric. Exp. Sta.

[3]Correspondence: ADM-MoorMan's, Inc., 1000 N. 30th St., Quincy, IL 62305 (phone: 217-231-2751; fax: 217-222-1794; E-mail: hyang@moorman.com).

Received March 11, 1999.

Accepted October 4, 1999.

quired for maximal lactation performance in primiparous sows has shortened the weaning-to-estrus interval (Wilson et al., 1996) and either increased (Tritton et al., 1996) or decreased second litter size (Touchette et al., 1998, Yang et al., 2000b). We postulated that altered reproductive performance might be associated with an altered metabolic state due to very high lysine intake during lactation. Therefore, we investigated the effects of a very low (**LL**), a medium (**ML**) and a very high (**HL**) dietary lysine concentration during lactation on components of metabolic state (blood metabolites and metabolic hormones), protein metabolism, reproductive hormones, and postweaning reproductive performance.

Materials and Methods

Animals, Dietary Treatments, and Management. Estrous cycles of 36 gilts (PIC Camborough 22) were synchronized with daily oral administration of 15 mg/gilt of allyl-trenbolone (Regumate, Roussel-UCLAF, Paris, France) for 14 d. At the first estrus following the last feeding of allyl-trenbolone, gilts were inseminated artificially twice per day for each day they exhibited estrus with pooled semen from purebred Hampshire boars. During gestation, gilts were housed in individual stalls (.6 × 2.1 m) and fed approximately 1.8 kg/d of a corn-soybean meal diet containing 3.2 Mcal ME/kg and 14% CP (.68% lysine). Between d 107 and 110 of gestation, gilts were fitted nonsurgically with indwelling jugular vein cannulas to facilitate blood collection during lactation. Immediately after cannulas were installed, gilts were moved to an environmentally controlled farrowing room and housed in individual farrowing stalls (1.3 × 2.0 m) until weaning. The minimum air temperature in the farrowing room was set at 20°C. Sows were provided with drip cooling when air temperature exceeded 25°C. Water was available to each gilt ad libitum from a nipple drinker. Water flow rate from all waterers was greater than 1,000 mL/min with an average rate of 1,300 mL/min. The experiment was conducted in three replicates (12 gilts/replicate) from March to August 1997. Sows farrowed between 10 and 12 mo of age with an average postfarrowing weight and backfat depth of 175.5 ± 3.59 kg and 15.2 ± .79 mm (6 cm off midline at the 10th rib; P2).

On d 110 of gestation, gilts were assigned according to body weight and backfat thickness to one of the three dietary treatments (LL, ML, or HL) consisting of .4, 1.0, or 1.6% lysine (Table 1). After farrowing, gilts were offered 5.0 kg/d of treatment diets to achieve target lysine intakes of about 20, 50, and 80 g/d. All other essential amino acids were provided at a minimum of 105% of the NRC (1988) recommended ratio to the lysine requirement to ensure that lysine was the first-limiting amino acid in all diets. Using the NRC (1998) lactating sow model (assuming 15 kg of sow body weight loss and daily weight gain of litters at 175 g/pig during lactation), the amount of other essential amino acids provided by all experimental diets was at least 10% higher than the ratio to the lysine requirement suggested by NRC (1998) based on analyzed amino acid concentrations (Table 1). Appropriate combinations of corn and soybean meal were used to achieve dietary lysine contents. Corn gluten meal was included in all experimental diets due to its low lysine:valine ratio to minimize supplementation of synthetic valine. The ratios of other essential amino acids to lysine varied across diets. With the increase of lysine concentrations in the diets, crude protein increased across the three diets. The responses to the highest lysine (1.6%) diet may result from a combination of lysine and other amino acids (or protein effects).

The calculated NE content (2,094 kcal/kg) was similar for all experimental diets. Net energy of corn, soy-

Table 1. Composition and analysis of experimental diets (as fed basis)

	Dietary lysine concentration during lactation		
Ingredient (%)	LL	ML	HL
Dent yellow corn	70.91	54.02	37.67
Soybean meal, 46%	5.46	27.02	48.16
Rice hulls	9.50	5.00	0.00
Soybean oil	5.00	5.00	5.00
Corn gluten meal, 60%	5.00	5.00	5.00
Dicalcium phosphate	2.40	2.21	1.99
Limestone	.83	.82	.83
Salt	.50	.50	.50
Vitamin premix[a]	.30	.30	.30
Trace mineral premix[b]	.10	.10	.10
Valine	—	.03	.27
DL-Methionine	—	—	.10
L-Threonine	—	—	.08
Total	100.00	100.00	100.00
Calculated analysis, % of diet			
Crude protein	11.90	20.26	28.82
Total lysine	.40	1.00	1.60
NE, kcal/kg	2,095	2,092	2,095
Crude fat	8.07	8.06	8.05
Starch	54.17	48.73	43.55
Calcium	1.00	1.00	1.00
Available phosphorus	.49	.49	.49
Analyzed content, % of diet			
Crude protein	11.75	19.13	28.40
Calcium	1.10	1.00	1.10
Phosphorus, total	.90	1.00	.90
Arginine	.55	1.21	1.80
Histidine	.31	.54	.74
Leucine	1.50	2.04	2.53
Lysine	.40	.99	1.50
Methionine/cystine	.48	.69	.99
Phenylalanine	.63	1.07	1.45
Threonine	.42	.76	1.14
Tryptophan	.13	.22	.33
Valine	.58	.98	1.50

[a]Supplied per kilogram of final diet: 6,600 IU vitamin A; 1,650 IU vitamin D_3; 27.5 IU vitamin E; 4.4 mg vitamin K (menadione sodium bisulfite); 6.6 mg riboflavin; 40 mg niacin; 17.6 mg D-pantothenic acid; 33 μg vitamin B_{12}; .88 mg pyridoxine; 1.1 mg folic acid; 198 μg biotin; .7 mg thiamin, and .6 g choline.

[b]Supplied per kilogram of final diet: 100 mg Zn; 100 mg Fe; 30 mg Mn; 6.6 mg Cu; .6 mg I, and .1 mg Se.

bean meal, and soybean oil (Ewan, 1991) was used to formulate diets. Net energy of rice hulls was assumed to be zero, as reported for dairy cattle (NRC, 1989). Net energy of corn gluten meal was calculated from the following equation (Noblet et al., 1994): NE = .730 (ME) – .0028 (CP) + .0055 (ether extract) – .0041 (crude fiber) + .0015 (starch). Because no starch data were available for corn gluten meal, nitrogen-free extract replaced starch in the equation. Rice hulls were used to equalize NE concentrations across experimental diets. We chose to add fiber rather than fat to equalize NE across the experimental diets because varying the proportion of dietary energy from fat may affect reproductive performance of sows (Kemp et al., 1995). Equal concentrations of vitamins and minerals were provided in all diets and were at least 125% of estimated requirements (NRC, 1988).

Litters were standardized to 10 pigs by d 3 of lactation. Pigs that died during lactation were replaced with pigs of comparable weight and age. Sows and their litters were weighed on d 0, 6, 12, and 18 of lactation. Sow backfat thickness at the P2 location was determined ultrasonically every 6 d throughout lactation (Leanmeater, Renco Corporation, Minneapolis, MN). Litters were weaned at 2100 on d 18 after farrowing. Sows were moved to gestation crates after weaning and were fed 2.7 kg/d of the gestation diet detailed above (3.2 Mcal ME/kg, 14% CP, .68% lysine). Sows were provided fence-line exposure to two boars for 15 min at 0900 and 1700 beginning the day after weaning and slaughtered during observed proestrus (equivalent to d 20 of the estrous cycle, before onset of the preovulatory LH surge, and approximately the same time relative to ovulation). Onset of proestrus and time of slaughter was determined by a team of experienced stockpersons based on behavioral and vulval characteristics. Behaviorally, sows responded positively to the presence of the boar, but a standing reflex to applied back pressure could still not be elicited. Reddening and swelling of the vulva and the appearance of vaginal mucus were also used as important criteria to establish onset of proestrus. Sows were weighed and scanned for backfat thickness at slaughter. Time of slaughter was determined so that preovulatory follicular fluid would be recovered from all sows at a similar stage of follicular development for determination of follicular quality (Yang et al., 2000a). Three sows from each treatment were excluded from analysis of data collected at proestrus to ensure that sows involved in measurement of follicular quality were slaughtered at a similar physiological stage (Yang et al., 2000a). These nine sows were included in analysis of data collected before proestrus.

This experiment was approved by an Institutional Animal Care and Use Committee at the University of Minnesota. Animals were cared for in accordance with guidelines outlined by the same committee.

Blood Sample Collection and Processing. Blood samples (10 mL) for serum LH analyses were collected at 15-min intervals for 8 h from 1300 to 2100 on d 12 and 18 postfarrowing. Blood (6 mL) for serum estradiol analysis was obtained at 1500 and 1900 on d 12 and 18 of lactation. After an overnight period without feed (a minimum of 15 h), blood samples for insulin (5 mL) and glucose (6 mL) analysis were obtained before the morning feeding at 0830 and at 25, 50, and 75 min after feeding on d 6, 12, and 18 of lactation. Blood samples (5 mL) were collected before the morning feeding and at 75 min after feeding for serum urea nitrogen (**SUN**) analysis or before the morning feeding and at 1300 for IGF-I analysis on d 6, 12, and 18 of lactation. At the time of slaughter, single blood samples for insulin, IGF-I, SUN, and estradiol analyses were collected. Blood samples for glucose were ejected immediately into tubes containing NaF to inhibit glycolysis and coagulation, were centrifuged at 1,520 × *g* for 20 min, and plasma was harvested. Blood samples for LH, estradiol, insulin, SUN, and IGF-I were allowed to clot at 4°C and centrifuged at 1,520 × *g* for 20 min before harvesting serum. Serum and plasma samples were stored at –20°C until assayed.

Glucose, Insulin, Urea Nitrogen, and IGF-I Assays. Plasma concentrations of glucose were analyzed using a commercial colorimetric kit (Sigma Chemical, St. Louis, MO) that is based on the glucose oxidase method. The only modification from the manufacturer's protocol was that standard curves were used instead of a single calibrator. The interassay CV of glucose for one plasma control (90 mg/dL) measured in 20 assays was 4.12%. Samples with an intraassay CV > 5% were reassayed. Samples with glucose concentrations exceeding the highest standard (150 mg/dL) were diluted with distilled, deionized water and reassayed. Insulin was measured with a solid-phase RIA (Diagnostic Products Corporation, Los Angeles, CA) as modified by Knudson (1990). Concentrations of the low, medium, and high serum controls were 8.57, 22.23, and 67.33 μIU/mL, respectively. The intraassay CV for these serum controls were 13.7, 3.7, and 4.2%, respectively, and the interassay CV (12 assays) were 15.4, 6.6, and 10.1%, respectively. A commercial colorimetric kit (Sigma Chemical Co.) was used to measure SUN concentration. The interassay CV of SUN for one serum control (13.7 mg/dL) was 1.15%, and the intraassay CV (12 assays) was 2.95%. Serum IGF-I concentrations were analyzed in triplicate (Frey et al., 1994), following an extraction of serum using .1 *M* glycyl glycine in a ratio of 1:1. Sensitivity of the assay, defined as 90% of total binding, was 24.4 ng/mL. Interassay CV for low (52.9 ng/mL), medium (104.4 ng/mL), and high (214.9 ng/mL) serum controls were 10.2, 2.4, and 2.4% and the intraassay CV were 13.4, 5.3, and 2.8%.

Serum Estradiol-17β Assay. Estradiol-17β (E_2) was extracted from 1 mL of serum by the addition of 5 mL of diethyl ether and vortexing for eight one-min pulses. Serum samples obtained at proestrus were prediluted fourfold with assay buffer (PBS containing .1% gel, pH 7.0), and sera collected during lactation were not diluted. Extraction tubes were then placed in a liquid

nitrogen/methanol bath, and the aqueous layer was allowed to freeze. The solvent supernatant was decanted and dried under vacuum. The average extraction efficiency was 75%, and estimated potencies were not corrected for recovery. Estradiol concentrations were estimated in a single RIA using a double antibody kit (Diagnostics Products Corp.) with the following modifications. In-house estradiol standards in assay buffer were used rather than the standards provided by the kit. The kit's first antibody was reconstituted as directed by the manufacturer and further diluted threefold with distilled, deionized water to improve assay sensitivity. Serial dilution of a serum control produced good parallelism (22.59, 11.31, 5.17, 3.33, and 1.59 pg/mL of estradiol, respectively). The intraassay CV was 8.03%. The assay sensitivity, defined as 90% of total binding, was .35 pg/mL.

Serum Luteinizing Hormone Assay. Serum LH concentrations were measured using a heterologous double antibody RIA (Dial et al., 1983). Purified porcine LH (AFP 10714B) was radioiodinated by reaction with ^{125}I (NEN, catalog number NEZ-033L; Boston, MA) in the presence of chloramine-T (catalog number C9887, Sigma Chemical Co.), and diluted in PBS containing .1% gelatin (pH 7.0) to give 25,000 counts per minute (cpm)/.1 mL. Each serum sample (300 μL) was assayed in duplicate. A purified porcine reference standard (LER 783-3) was used to express the results. The assay sensitivity, defined as 90% of total binding, was .17 ng/mL. The intraassay CV for low (.32 ng/mL), medium (1.17 ng/mL), and high (2.00 ng/mL) LH reference pools were 10.2, 4.6, and 3.3%, respectively. The interassay CV (n = 9) for low, medium, and high LH control sera were 10.3, 7.0, and 6.6%, respectively. Characteristics of pulsatile patterns of LH secretion were determined using a peak detection program (CLUSTER) for microcomputers (Veldhuis and Johnson, 1988). Two points of nadir and one point for peak with a 5% false-positive rate were used to detect number of pulses and mean peak height of pulses.

Estimation of Sow Body Protein Breakdown (3-Methylhistidine Production). Muscle myofibrillar protein breakdown was determined from a kinetic analysis of 3-methylhistidine (**3MH**) metabolism on d 4 and 15 postpartum using the method of Rathmacher et al. (1996). Thirty minutes after the morning meal, each gilt was infused with a bolus dose (.1371 mg/kg BW) of [methyl-$^2H_3$]-3MH in 10 mL of sterile saline via the jugular catheter (in 10 s). After infusion, the catheter was flushed with an additional 10 mL of sterile saline. Serial 10-mL blood samples were taken at –1, 2, 5, 15, 60, 120, 180, 360, 540, 720, 1,440, 2,160, 2,880, and 3,600 min following the bolus infusion to measure the disappearance of stable isotope in plasma. Blood samples were transferred to glass tubes coated with EDTA and placed on ice. Thirty minutes after collection, blood samples were centrifuged at 500 × *g* for 20 min, and the plasma was harvested and stored at –20°C for later analysis of 3MH with gas chromatography/mass spectrometry using the method of Rathmacher et al. (1992). The de novo production rate of 3MH (μmol/d), fractional breakdown rate of muscle, and fat-free muscle mass were estimated according to Rathmacher et al. (1992).

Statistical Analyses. All dependent variables were checked for normality using the Wilk-Shapiro test (SAS, 1990). Repeated measures ANOVA using the GLM procedures of SAS (1990) was used to analyze experimental data except weaning-to-proestrus interval. Concentrations of metabolites and metabolic hormones measured at slaughter were not included in the repeated measures analysis because of the differences in feeding regimens, sampling frequency, and the physiological status of the sows after weaning. The statistical models included dietary treatment, replicate, stage of lactation (day), sampling time (within day), and their interactions. Weaning-to-proestrus interval was transformed logarithmically to normalize the data and then analyzed by simple ANOVA of SAS (1990). Three preplanned contrasts (LL vs ML, LL vs HL, and ML vs HL) were used to separate treatment means when the *F*-test was significant. Pearson correlation coefficients were used to evaluate relationships between metabolite concentrations, metabolic hormone concentrations, or fractional breakdown rate of muscle and LH secretion.

Results

Sow and Litter Performance. Feed intake during lactation was less than the target amount (5 kg/d) and averaged about 73% of the feed offered (Table 2). With the increase in dietary lysine concentration, ADFI of sows decreased at all stages of lactation. Daily feed intakes during d 13 to 18 and throughout (d 1 to 18) lactation were higher ($P < .05$) for sows fed LL than for sows fed HL. Total BW loss was greater ($P < .01$) during lactation but lower ($P < .05$) from weaning to slaughter for sows fed LL compared with sows fed HL (Table 2). Dietary lysine concentration did not affect overall change in backfat during lactation and from weaning to slaughter, nor did it affect weaning-to-proestrus interval. Low lysine intake during lactation decreased overall litter weight gain ($P < .05$) compared with medium and high lysine intake. However, litter weight gain was similar for litters nursing sows fed ML or HL.

Metabolites and Metabolic Hormones. Preprandial plasma glucose (Table 3) and serum insulin (Table 4) concentrations decreased ($P < .01$), but mean postprandial glucose and insulin levels increased ($P < .01$) as lactation advanced. Glucose and insulin concentrations were higher ($P < .01$) after than before feeding when sows were deprived of feed overnight. Dietary lysine intake was associated positively ($P < .05$) with mean postprandial serum insulin concentrations but did not affect pre- and postprandial plasma glucose concentrations and preprandial insulin levels on d 6, 12, and 18 after farrowing and insulin levels at postweaning proestrus.

Table 2. Effects of dietary lysine intake during lactation on sow and litter performance

	Dietary lysine during lactation			
	LL	ML	HL	SE
Number of sows	12	12	12	—
Initial body weight, kg	177.0	176.7	172.7	3.59
Initial backfat depth, mm	15.1	14.9	15.5	.79
Lysine intake, g/d	16.0	36.0	56.0	1.12
Feed intake, kg/d				
d 1 to 6	3.39	2.83	2.59	.40
d 7 to 12	3.88	3.76	3.41	.21
d 13 to 18	4.53^{w}	4.13	4.05^{x}	.17
d 1 to 18	3.93^{w}	3.57	3.45^{x}	.21
Sow weight change, kg				
d 1 to 6	–7.66	–9.03	–5.87	1.80
d 7 to 12	–7.85^{w}	–4.57	–3.76^{x}	1.23
d 13 to 18	–6.61	–5.00	–5.74	1.26
d 1 to 18	–22.13^{y}	–18.60	–15.37^{z}	1.65
Weaning to slaughter	–.56^{w}	–1.13	–4.50^{x}	1.28
Sow backfat change, mm				
d 1 to 6	–.79^{w}	–1.48^{xy}	–.35^{wz}	.24
d 7 to 12	–.92	–.14	–.34	.33
d 13 to 18	.35^{y}	–.53^{wz}	–1.33^{xz}	.23
d 1 to 18	–1.36	–2.16	–2.03	.33
Weaning to slaughter	–.44	–.23	–.28	.35
Weaning-to-proestrus interval, d[a]	4.6	4.9	4.6	.13
Litter weight gain, kg				
Initial litter wt	15.29	15.58	16.53	.47
d 1 to 6	8.32	9.23	8.49	.63
d 7 to 12	10.16^{w}	11.17	11.71^{x}	.54
d 13 to 18	10.43^{w}	12.18^{x}	12.56^{x}	.39
d 1 to 18	28.92^{w}	32.58^{x}	32.76^{x}	1.12

[a]Nine sows per treatment.

Means within a row with superscripts differ as follows: $^{wx}P < .05$; $^{yz}P < .01$.

Concentration of SUN (Table 5) was higher after than before feeding ($P < .01$). Both preprandial and postprandial SUN concentrations increased linearly ($P < .01$) with dietary lysine intake on d 6, 12, and 18 of lactation. However, SUN concentrations were similar among sows assigned to the three treatments at proestrus. Sampling time within a day did not affect IGF-I concentrations (data not shown). Mean serum IGF-I was greater ($P < .05$) on d 6 and 18 of lactation for sows fed HL compared with sows fed ML (Table 6). Serum IGF-I was higher ($P < .01$) at proestrus than during lactation for all treatments, but it was similar across the three treatment groups at proestrus when all sows consumed the same gestation diet after weaning.

Reproductive Hormones. Serum estradiol concentrations on d 18 of lactation were less ($P < .05$) for sows fed LL compared with ML and HL treatments, but there was no difference between sows fed ML and HL (Table 6). Serum estradiol concentrations were similar across the three treatments at proestrus when the sows consumed the same gestation diet after weaning. Estradiol concentrations were greater during proestrus than during lactation ($P < .01$). The number of LH peaks/8 h on d 12 ($P < .05$) and 18 ($P < .01$) of lactation was reduced for sows fed LL compared with sows fed ML and HL (Table 7). However, number of LH peaks was similar between sows fed ML and HL. As lactation advanced, the number of LH peaks increased ($P < .05$).

Protein Metabolism. Fractional breakdown rate of muscle decreased linearly ($P < .01$) on d 15 of lactation when dietary lysine intake increased, although there were no differences on d 4 (Table 8). Dietary lysine intakes during lactation had no effect on fat-free muscle mass. As lactation advanced, fat-free muscle mass ($P < .01$) and production of 3MH ($P < .1$) declined. Sows fed HL produced less 3MH ($P < .05$) on d 15 of lactation than those fed LL.

Correlation. Correlation between metabolites, metabolic hormones, or fractional breakdown rate of muscle and LH characteristics was examined. Serum insulin concentration at 25 min after feeding on d 6 was correlated ($P < .05$) with number of LH peaks/8 h on d 12 and 18 of lactation (Table 9). Insulin concentration at 25 min after feeding on d 18 was also correlated positively ($P < .01$) with number of LH peaks on d 18. Preprandial and postprandial SUN concentrations on d 6, 12, and 18 were correlated positively ($P < .05$) with number of LH peaks on d 12 and 18. The correlation between SUN and number of LH peaks seemed stronger than that between insulin and number of LH peaks. No other correlations were detected ($P > .05$).

Table 3. Effects of lysine intake during lactation on plasma glucose concentrations (mg/dL) of primiparous sows

	Lysine intake during lactation				
Trait	LL	ML	HL	Mean	SE
Number of sows	12	12	12	—	—
d 6 postfarrowing					
Preprandial[a]	73.1	69.5	72.2	71.6	4.12
Postprandial[b]					
25 min	89.1	85.8	86.8	87.6	3.56
50 min[c]	100.1	100.3	105.4	102.1	4.60
75 min[c]	96.2	96.0	107.5	100.2	5.93
Mean[c]	95.1	94.0	99.9	96.6	3.66
d 12 postfarrowing					
Preprandial	60.4	57.9	62.5	60.3	2.76
Postprandial					
25 min	86.2	79.6	82.4	82.8	5.20
50 min	101.5	102.7	102.5	102.3	6.63
75 min	97.2	100.3	106.2	101.3	8.01
Mean	94.9	94.2	97.0	95.5	6.16
d 18 postfarrowing					
Preprandial	54.6	53.3	58.7	55.7	2.12
Postprandial					
25 min	85.1	83.3	90.9	86.4	4.28
50 min	109.2	115.7	114.3	113.0	5.47
75 min	107.8	116.8	119.8	114.8	8.73
Mean	100.7	105.3	108.3	104.7	4.96

[a]Linear effect (decrease) of stage of lactation on preprandial glucose level, $P < .01$.

[b]Blood samples were collected at 25, 50, and 75 min after the morning feeding (sows were subject to an overnight period without feed of at least 15 h before the morning feeding).

[c]Linear effect (increase) of stage of lactation on postprandial glucose level, $P < .05$.

Table 4. Effects of lysine intake during lactation on serum insulin concentrations (μIU/mL) of primiparous sows

	Lysine intake during lactation				
Trait	LL	ML	HL	Mean	SE
Number of sows	12	12	12	—	—
d 6 postfarrowing[a]					
Preprandial[b]	9.14	8.81	13.14	10.59	2.45
Postprandial					
25 min	33.00	33.58	41.00	35.52	6.46
50 min	57.17	54.08	86.13	65.86	14.51
75 min[c]	40.09[u]	66.23	87.66[v]	65.11	11.70
Mean[d]	43.42[w]	51.60	71.60[x]	55.50	8.98
d 12 postfarrowing					
Preprandial	6.64	6.75	7.66	7.02	.89
Postprandial					
25 min	31.93	33.11	49.83	38.18	8.37
50 min	54.03[uy]	73.52[y]	104.34[vz]	75.86	11.95
75 min	45.33[u]	80.07[uw]	126.07[vx]	81.27	12.30
Mean	43.76[u]	62.24[uw]	93.41[vx]	65.11	8.90
d 18 postfarrowing					
Preprandial	6.04	5.78	8.13	6.65	.65
Postprandial					
25 min	29.19[y]	36.67	48.23[z]	38.19	7.35
50 min	63.39	97.16	92.33	84.64	14.14
75 min	60.05[u]	93.68	120.13[v]	92.48	13.53
Mean	50.88[w]	75.84	86.89[x]	71.77	9.71
Proestrus[e]	12.03	19.72	20.14	17.30	3.83

[a]Preprandial is one sample taken shortly before morning feeding after an overnight period without feed of at least 15 h; one sample was taken at 25, 50, and 75 min after feeding, respectively.
[b]Linear effect (decrease) of stage of lactation on preprandial insulin level, $P < .01$.
[c]Linear effect (increase) of stage of lactation on serum insulin level 75 min after feeding, $P < .05$.
[d]Linear effect (increase) of stage of lactation on postprandial insulin level, $P < .1$.
[e]Nine sows per treatment.
Means within a row with superscripts differ as follows: [uv]$P < .01$; [wx]$P < .05$; [yz] $< .1$.

Discussion

Compared with medium lysine intake, low lysine intake decreased litter growth rate and tended to increase the loss of sow body weight. This is consistent with the previous finding that sows will mobilize body reserves to support milk production when nutrient intake is inadequate (Tokach et al., 1992). The mobilized body reserves might be body protein. Low lysine intake did not affect backfat loss significantly but tended to increase muscle protein degradation, suggesting that dietary protein was insufficient to support milk production. Similarly, Jones and Stahly (1999b) reported that protein mobilization as evidenced by fractional breakdown rate of muscle was increased for sows fed inadequate lysine during lactation. Weaning-to-proestrus interval (mean of 4.7 d) was not influenced by dietary lysine. Proestrus is about 1.5 d before estrus (Rojkittikhun et al., 1992); thus, weaning-to-estrus interval might have been 6.2 d in this study, and is comparable to that reported in a companion study (Yang et al., 2000b). However, sows fed LL in this study had lower lysine intake (16 g/d) than that of sows fed the diet containing the lowest lysine (33 g/d) in the companion study. King (1991) suggested that sows with lysine intake below 35 g/d experienced a prolonged weaning-to-estrus interval. Thus, low lysine intake should be expected to elicit a protracted weaning-to-proestrus interval compared with higher lysine intakes in the present study. Lack of response might be due to limited numbers of animals per treatment and due to the fact that proestrus may not be a distinctive point in time. It is also possible that low lysine intake did not affect weaning-to-proestrus interval but prolonged the duration of proestrus. Rojkittikhun et al. (1992) reported a longer duration of proestrus for sows with a longer weaning-to-estrus interval. Because sows were slaughtered at proestrus, actual weaning-to-estrus interval could not be determined in this study.

Inadequate nutrient intake during lactation causes the sow to become catabolic. When lysine intake decreased from 36 to 16 g/d, postprandial serum insulin concentrations were reduced, which is consistent with previous reports that restricting lysine (Tokach et al., 1992; Kusina, 1995) or energy intake (Koketsu et al., 1996; Zak et al., 1997) decreased serum insulin concentration. Tokach et al. (1992) demonstrated that LH secretion was reduced by restriction of either lysine or energy intake. Our data confirm that low lysine intake reduced LH pulses during mid- and late lactation compared with medium and high lysine intake. Armstrong and Britt (1987) suggested that inadequate nutrient intake during lactation might inhibit either the release of LH from the pituitary or suppress the hypothalamic GnRH pulse generator.

Estradiol is produced primarily by ovarian follicles. Sows fed LL had lower serum E_2 concentration during late lactation than sows fed ML and HL, suggesting that

Table 5. Effects of dietary lysine intake during lactation on serum urea nitrogen concentration (mg/dL) of primiparous sows

	Lysine intake during lactation			
Trait	LL	ML	HL	SE
Number of sows	12	12	12	—
d 6 postfarrowing[a]				
Preprandial	11.82[w]	18.51[x]	21.96[y]	.97
Postprandial	12.37[w]	19.83[x]	23.49[y]	1.21
d 12 postfarrowing				
Preprandial	11.58[w]	17.07[x]	20.65[z]	1.05
Postprandial	12.04[w]	18.29[x]	22.35[z]	1.07
d 18 postfarrowing				
Preprandial	13.12[w]	17.76[x]	21.17[z]	1.20
Postprandial	12.76[w]	19.60[x]	25.42[z]	1.48
Proestrus[b]	9.69	11.07	10.53	.73

[a]Preprandial was one sample taken shortly before the morning feeding after an overnight fast of at least 15 h; postprandial was one sample taken at 75 min after the morning feeding.
[b]Nine sows per treatment.
Means within a row with superscripts differ as follows: [wx, wy, wz, xz]$P < .01$; [xy]$P < .05$.

Table 6. Effects of dietary lysine intakes during lactation on serum IGF-I (ng/mL) and estradiol (pg/mL) concentrations of primiparous sows

	Lysine intake during lactation			
Item	LL	ML	HL	SE
Number of sows	12	12	12	—
		IGF-I		
d 6 of lactation	181.9	145.6^{w}	223.2^{x}	21.41
d 12 of lactation	168.2	169.4	194.8	21.82
d 18 of lactation	157.5^{w}	143.7^{w}	197.3^{x}	16.83
Proestrus[a]	274.8	289.9	295.1	19.40
		Estradiol		
d 12 of lactation	3.09	3.37	3.18	.24
d 18 of lactation	2.67^{y}	3.94^{z}	3.98^{z}	.34
Proestrus[a]	35.01	32.34	33.70	6.35

[a]Nine sows per treatment.
Means within a row with superscripts differ as follows: $^{wx}P < .05$; $^{yz}P < .01$.

nutrient intake could modulate follicular development during lactation and beyond. This phenomenon has been described as the nutritional "imprinting" of follicles by Cosgrove and Foxcroft (1996). Direct evidence was provided by the observation that low lysine intake reduced the proportion of large follicles at proestrus in our companion study (Yang et al., 2000a). However, results presented herein did not show effects of dietary treatments on concentration of serum estradiol at proestrus. Our blood sample for proestrus E_2 measurement was taken immediately after slaughter. Slaughter is a stress to the sow and may have stimulated E_2 release from the adrenal cortex. Brown et al. (1959) found that the adrenal gland is the major source of synthesis and release of estrogens during stress in women. Thus, E_2 from the adrenal glands in our sows may have contributed significant amounts to the total concentration of serum E_2 collected at slaughter. In the present study, serum E_2 at proestrus may not provide a true picture of ovarian function.

Although many studies have been conducted to evaluate the effects of low lysine intake on metabolic state and reproductive hormone secretion, few studies have been conducted to evaluate the effects of relatively high lysine intake. Compared with medium lysine intake, high lysine intake increased postprandial concentrations of serum insulin and IGF-I. Increased concentrations of insulin with increased lysine intake during lactation agree with the conclusion of Murray et al. (1988) that insulin secretion is associated positively with protein intake. Increasing protein intake increases amino acid concentrations in both portal and peripheral circulation (Rerat et al., 1988) and, thus, stimulates release of insulin in nonruminant animals (Unger, 1974). Increasing insulin causes up-regulation of liver GH receptors, thereby stimulating hepatic IGF-I production (I'Anson et al., 1991). These increased concentrations of serum insulin and IGF-I suggest an improved metabolic state with high lysine intake.

Very high lysine intake during the first lactation shortened weaning-to-estrus interval (Wilson et al., 1996) and increased second litter size (Tritton et al., 1996) in sows. This improved reproductive performance is assumed to be associated with an increased secretion of reproductive hormones. However, high lysine intake did not further stimulate secretion of LH and estradiol compared with medium lysine intake in the present study, supporting our finding that increasing lysine intake from 33 to 54 g/d in primiparous sows did not increase subsequent litter size (Yang et al., 2000b). Previous research from our group (Tokach et al., 1992) suggested that the influence of lysine intake on LH secretion increased as energy intake increased. In the present study, feed intake of sows was low; thus, energy intake for sows with the highest lysine intake may not have been sufficient to allow the high lysine intake to stimulate LH and estradiol secretion. We suspect that the intensive manipulation limited the feed intake for all experimental sows and caused decreased milk production compared with sows on well-managed commercial farms, as evidenced by ADG of 1.75 kg for a 10-pig litter over an 18-d lactation. In our companion study with a similar sow genotype and dietary formulations, litter ADG was 2.00 kg/d (Yang et al., 2000b). Furthermore, voluntary feed intake of sows declined as dietary lysine increased. A similar response was observed in a companion study (Yang et al., 2000b) and possible explanations have been offered by Yang et al. (2000b).

Loss of body protein during lactation may influence subsequent reproduction of sows. Reducing protein loss during lactation reduced weaning-to-estrus interval (King, 1987). Our high lysine intake did not decrease fractional breakdown rate of muscle compared with ML, suggesting that very high lysine intake did not further reduce loss of body protein. Correlation analysis between fractional breakdown rate of muscle and LH characteristics failed to reveal that LH secretion was

Table 7. Effects of dietary lysine intake on serum LH characteristics in primiparous lactating sows

	Lysine intake during lactation			
	LL	ML	HL	SE
Number of sows	12	12	12	—
No. of LH peaks/8 h				
d 12 of lactation	.94^{w}	1.75^{x}	1.68^{x}	.29
d 18 of lactation	1.19^{y}	2.0^{z}	2.23^{z}	.21
Mean LH concentrations, ng/ml				
d 12 of lactation	.40	.40	.37	.02
d 18 of lactation	.40	.41	.38	.02
LH peak height, ng/ml				
d 12 of lactation	.66	.65	.61	.08
d 18 of lactation	.61	.64	.62	.04

Means within a row with superscripts differ as follows: $^{wx}P < .05$; $^{yz}P < .01$.

correlated with protein mobilization. Collectively, these results indicate that feeding a very high lysine diet may not further improve body protein metabolism and subsequent reproductive traits such as weaning-to-estrus interval and litter size.

Pulsatile secretion of LH is an important factor in stimulating follicular development and resumption of estrus postweaning (Shaw and Foxcroft, 1985; King and Martin, 1989). Furthermore, LH pulse frequency during mid- to late lactation was related to weaning-to-estrus interval (Tokach et al., 1992; Koketsu et al., 1996). Our correlation analysis revealed a positive association between LH pulses and SUN (both preprandial and postprandial) concentrations during lactation, suggesting that dietary treatment effects on LH characteristics may be mediated through effects on SUN concentrations, as reported previously by Kusina (1995). However, this connection between SUN and LH seems unlikely from a physiological standpoint. We are not aware of any physiological mechanism by which high SUN concentrations would stimulate LH release. Thus, we can not confidently propose a causal relationship between SUN and LH release, although both responded to increased lysine intake.

In the present study, only insulin concentration at 25 min after feeding was correlated positively with number of LH peaks. Tokach et al. (1992) and Koketsu et al. (1996) demonstrated that concentration of serum insulin during lactation is associated positively with LH release and weaning-to-estrus interval, and this suggested that dietary influences on reproduction may be mediated in part through associated effects on circulating insulin. Dietary treatments among the four recent studies from our laboratory that have addressed this issue have differed. This study and the study of Kusina et al. (1999) varied dietary protein and lysine concentration, Koketsu et al. (1996) varied dietary energy, and Tokach et al. (1992) altered both dietary energy and lysine. A stronger correlation may exist between insulin and LH release when energy intake varies than when amino acid intake varies. Some studies have implicated glucose (Koketsu et al., 1996) or IGF-I (Pettigrew and Tokach, 1993; Booth et al., 1994) as a key component in the connection between diet and reproduction. However, we did not find any correlation between circulating glucose or IGF-I and LH characteristics. Therefore, the results of this study show that effects of lysine intake on LH characteristics may not be mediated through effects on concentrations of circulating glucose or IGF-I.

Table 8. Effects of dietary lysine intake during lactation on protein metabolism of primiparous sows

	Lysine intake during lactation			
	LL	ML	HL	SE
Number of sows	12	12	12	—
Fat-free muscle mass, kg[a]				
d 4 of lactation	84.36	85.48	88.34	3.04
d 15 of lactation	75.12	78.37	74.33	2.75
3-methylhistidine production, μmol·kg^{-1}·d^{-1}[b]				
d 4 of lactation	18.02	19.44	17.15	1.66
d 15 of lactation	17.34^{w}	16.20	13.43^{x}	1.28
Fractional breakdown rate of muscle, %/d				
d 4 of lactation	4.56	4.73	4.03	.31
d 15 of lactation	5.57^{w}	4.22^{y}	3.31^{z}	.54

[a]Stage of lactation effect on fat-free muscle mass, $P < .001$; [b]Stage of lactation effect on 3-methylhistidine production, $P < .1$.

Means within a row with superscripts differ as follows: $^{wx}P < .05$; $^{wy}P < .1$; $^{wz}P < .01$.

Table 9. Correlation coefficients relating blood urea nitrogen and serum insulin to number of LH peaks per 8 h during lactation[a]

	Number of LH peaks per 8 h	
Item	on d 12	on d 18
Serum insulin: 25 min after feeding		
d 6 of lactation	.31*	.41**
d 12 of lactation	.21	.12
d 18 of lactation	.32*	.37**
Preprandial serum urea nitrogen		
d 6 of lactation	.45***	.46***
d 12 of lactation	.33*	.53***
d 18 of lactation	.40**	.44**
Postprandial serum urea nitrogen		
d 6 of lactation	.38**	.56***
d 12 of lactation	.31*	.58***
d 18 of lactation	.30*	.44**

*$P < .1$; **$P < .05$; ***$P < .01$.

[a]Blood samples for insulin analysis were collected before and 25, 50, and 75 min after the morning feeding (sows were subject to an overnight period without feed of at least 15 h before the morning feeding). Blood samples for urea nitrogen analysis were collected before and 75 min after the morning feeding.

Implications

Low lysine intake during lactation seems to increase sow body protein mobilization, and increased lysine intake seems to improve the metabolic status of sows. Increasing lysine intake from 16 to 36 g/d increased luteinizing hormone (LH) pulses and estradiol secretion during lactation, but further increasing lysine intake to 56 g/d did not enhance secretion of reproductive hormones. This may indicate a lack of further improvement in follicular development and subsequent reproduction. Existence of a significant correlation between insulin and LH pulse frequency suggests that nutritional influences on LH release may be mediated, at least in part, through associated effects on circulating insulin concentration.

Literature Cited

Armstrong, J. D., and J. H. Britt. 1987. Nutritionally-induced anestrus in gilts: Metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. 65:508–523.

Booth, P. J., J. Craigon, and G. R. Foxcroft. 1994. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J. Anim. Sci. 72:2415–2425.

Brown, J. B., C. W. A. Falconer, and J. A. Strong. 1959. Urinary estrogens of adrenal origin in women with breast cancer. J. Endocrinol. 19:52–60.

Cosgrove, J. R., and G. R. Foxcroft. 1996. Nutrition and reproduction in the pigs: Ovarian aetiology. Anim. Prod. Sci. 42:131–141.

Dial, G. D., O. K. Dial, G. W. Bevier, S. D. Glenn, and P. J. Dzuik. 1983. Estrous behavior and circadian discharge of luteinizing hormone in the prepubertal gilt in response to exogenous estrogen. Biol. Reprod. 29:1047–1056.

Ewan, R. C. 1991. Energy utilization in swine nutrition. In: E. R. Miller, D. E. Ullrey and A. J. Lewis (Ed.). Swine Nutrition. pp 121–132. Butterworth-Heinemann, New York.

Frey, R. S., M. R. Hathaway, and W. R. Dayton. 1994. Comparison of the effectiveness of various procedures for reducing or eliminating insulin-like growth factor binding protein interference with insulin-like growth factor-1 radioimmunoassays on porcine sera. J. Endocrinol. 140(2):229–237.

I'Anson, H., D. L. Foster, G. R. Foxcroft, and P. J. Booth. 1991. Nutrition and reproduction. Oxf. Rev. Reprod. Biol. 13:239–311.

Jones, D. B., and T. S. Stahly. 1999a. Impact of amino acid nutrition during lactation on luteinizing hormone secretion and return to estrus in primiparous sows. J. Anim. Sci. 77:1523–1531.

Jones, D. B., and T. S. Stahly. 1999b. Impact of amino acid nutrition during lactation on body nutrient mobilization and milk nutrient output in primiparous sows. J. Anim. Sci. 77:1513–1522.

Kemp, B., N. M. Soede, F. A. Helmond, and M. W. Bosch. 1995. Effects of energy source in the diet on reproductive hormones and insulin during lactation and subsequent estrus in multiparous sows. J. Anim. Sci. 73:3022–3029.

King, R. H. 1987. Nutritional anestrus in young sows. Pig News Info. 8:15–22.

King, R. H. 1991. Nutrition of sows during lactation. In: Proc. of the 52nd Minnesota Nutrition Conference, Bloomington, MN. pp 154–162.

King, R. H., and G. B. Martin. 1989. Relationships between protein intake during lactation, LH levels and estrus activity in first-litter sows. Anim. Reprod. Sci. 19:283–292.

Knudson, B. J. 1990. Influence of bioenergetics upon metabolite levels, body composition and reproductive performance of female swine. Ph.D. dissertation. University of Minnesota, St. Paul.

Koketsu, Y., G. D. Dial, J. E. Pettigrew, W. E. Marsh, and V. L. King. 1996. Influence of imposed feed intake patterns during lactation on reproductive performance and on circulating levels of glucose, insulin, and luteinizing hormone in primiparous sows. J. Anim. Sci. 74:1036–1046.

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

Murray, P. K., D. K. Granner, P. A. Mayes, and V. W. Rodwell. 1988. Harper's Biochemistry (21st Ed.). Appleton and Lange, Norwalk, CT.

Noblet, J., H. Fortune, X. S. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72:344–354.

NRC. 1988. Nutrient Requirements of Swine (9th Ed.). National Academy Press, Washington, DC.

NRC. 1989. Nutrient Requirements of Dairy Cattle (6th Ed.). National Academy Press, Washington, DC.

NRC. 1998. Nutrient Requirements of Swine (10th Ed.). National Academy Press, Washington, DC.

Pettigrew, J. E., and M. D. Tokach. 1993. Metabolic influences on sow reproduction. Pig News Info. 14:69N–72N.

Quesnel, H., and A. Prunier. 1995. Endocrine bases of lactational anoestrus in the sow. Reprod. Nutr. Dev. 35:395–414.

Rathmacher, J. A., G. A. Link, and S. L. Nissen. 1992. Measurement of 3-methylhistidine production in lambs by using compartmental-kinetic analysis. Br. J. Nutr. 69:743–755.

Rathmacher, J. A., S. L. Nissen, R. E. Paxton, and D. B. Anderson. 1996. Estimation of 3-methylhistidine production in pigs by compartmental analysis. J. Anim. Sci. 74:46–56.

Rerat, A., J. Jung, and J. Kande. 1988. Absorption kinetics of dietary hydrolysis products in conscious pigs given diets with different amount of fish protein. 2. Individual amino acids. Br. J. Nutr. 60:105–120.

Rojkittikhun, T., M. Sterning, L. Rydhmer, and S. Einarsson. 1992. Estrous symptoms and plasma levels of estradiol-17b in relation to the interval from weaning to estrus in primiparous sows. In: 2nd Proc. of 12th International Pig Veterinary Society, p 485. The Hague, The Netherlands.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Shaw, H. J., and G. R. Foxcroft. 1985. Relationships between LH, FSH and prolactin and reproductive activity in the weaned sow. J. Reprod. Fertil. 75:17–28.

Tokach, M. D., J. E. Pettigrew, G. D. Dial, J. E. Wheaton, B. A. Crooker, and L. J. Johnston. 1992. Characterization of luteinizing hormone secretion in the primiparous, lactating sow: Relationship to blood metabolites and return-to-estrus interval. J. Anim. Sci. 70:2195–2201.

Touchette, K. J., G. L. Allee, M. D. Newcomb, and R. D. Boyd. 1998. The lysine requirement of lactating primiparous sows. J. Anim. Sci. 76:1091–1097.

Tritton, S. M., R. H. King, R. G. Campbell, A. C. Edwards, and P. E. Hughes. 1996. The effects of dietary protein and energy levels of diets offered during lactation on the lactational and subsequent reproductive performance of first-litter sows. Anim. Sci. 62:573–579.

Unger, R. H. 1974. Alpha- and beta- cell interrelationships in health and disease. Metabolism 23:581–593.

Veldhuis, J. D., and M. L. Johnson. 1988. Cluster analysis: A simple versatile and robust algorithm for endocrine pulse detection. Am. J. Physiol. 250:E486–E493.

Wilson, M. E., H. Stein, N. L. Trottier, D. D. Hall, R. L. Moser, D. E. Orr, and R. A. Easter. 1996. Effect of lysine intake on reproductive performance in first parity sows. J. Anim. Sci. 74(Suppl.1):63 (Abstr.).

Yang, H., G. R. Foxcroft, J. E. Pettigrew, L. J. Johnston, G. C. Shurson, A. N. Costa, and L. J. Zak. 2000a. Impact of dietary lysine intake during lactation on follicular development and oocyte maturation after weaning in primiparous sows. J. Anim. Sci. 78:993–1000.

Yang, H., J. E. Pettigrew, L. J. Johnston, G. C. Shurson, and R. D. Walker. 2000b. Lactational and subsequent reproductive responses of lactating sows to dietary lysine (protein) concentration. J. Anim. Sci. 78:348–357.

Zak, L. J., J. R. Cosgrove, F. X. Aherne, and G. R. Foxcroft. 1997. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J. Anim. Sci. 75:208–216.