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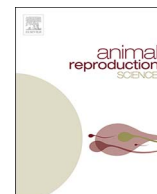
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Effect of feeding three lysine to energy diets on growth, body composition and age at puberty in replacement gilts[☆,☆☆]

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ABSTRACT

This study evaluated the effect of diets differing in standard ileal digestible (SID) lysine on lysine intake, growth rate, body composition and age at puberty on maternal line gilts. Crossbred Large White × Landrace gilts ($n = 641$) were fed corn-soybean diets differing in SID lysine concentration (%, g SID lysine:Mcal ME); diets were not isocaloric. Gilts received three grower, finisher diet combinations: low (0.68% lysine grower, 0.52% lysine finisher), medium (0.79% lysine grower, 0.60% lysine finisher) or high (0.90% lysine grower, 0.68% lysine finisher). Grower diets were fed from 100 until 142 days of age, and finisher diets were fed until they reached 220 days of age. Body weight (BW), backfat thickness (BF), and loin depth (LD) were recorded every 28 days. From 160–220 days of age, gilts were exposed daily to vasectomized boars and observed for behavioral estrus. Gilts fed the low lysine diet had lower average daily gain and BW ($P < 0.05$), but not fat depth:LD ratio. The percentage of gilts that displayed natural estrus by 220 days of age was low but not different among dietary treatments (low 27.7%, medium 31.0% and high 37.7%, respectively; $P = 0.1201$). Gilts fed the high and medium diets reached puberty 10 and 6 days earlier, however, than gilts fed the low lysine diet ($P < 0.05$). The rate of puberty attainment may have been less because all gilts contracted porcine epidemic diarrhea (PEDv) just as boar exposure was to begin for the first group of gilts. Results from the

present study indicate that growth rate and age at puberty can be altered by *ad libitum* fed diets that differ in SID lysine concentration.

1. Introduction

Proper gilt development is of vital importance to maximize sow reproductive performance and sow longevity. The Animal Science Committee of the National Pork Board commissioned studies to develop *ad libitum* fed gilt development diets that result in reduced

Table 1
Experimental grower and finisher diets composition used to feed to maternal line[a] gilts to evaluate three lysine concentrations and determine the effects on growth, body composition and age at puberty, as-fed basis.

Item	Grower diets			Finisher diets		
	Low lysine	Medium lysine	High lysine	Low lysine	Medium lysine	High lysine
Corn	48	58	73	49	66	80
De-oiled corn germ	16	8.0	0.0	20	7.5	0.0
Soy bean meal (47.25% CP)	14	14	17	7.5	7.5	10
Wheat middlings	18	15	5.0	18	15	5.0
Yellow grease	1.0	1.0	1.0	2.9	1.0	1.0
Limestone	0.98	0.92	0.76	0.93	0.90	0.77
Dicalcium phosphate (21%)	1.2	1.3	1.6	0.98	1.1	1.4
Liquid L-lysine (50%)	0.0	0.33	0.60	0.0	0.29	0.45
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Black iron oxide dye	0.0	0.0	0.25	0.0	0.0	0.25
Blue dye	0.01	0.0	0.0	0.01	0.0	0.0
Methionine hydroxy analogue 88% liquid	0.0	0.0	0.11	0.0	0.0	0.03
L-Threonine	0.0	0.04	0.14	0.0	0.02	0.09
Sow trace mineral premix[b]	0.18	0.18	0.18	0.18	0.18	0.18
Sow vitamin premix[c]	0.05	0.05	0.05	0.05	0.05	0.05
Biotin, 200 mg/l	0.03	0.03	0.03	0.03	0.03	0.03
L-tryptophan	0.0	0.0	0.03	0.0	0.0	0.02
Calculated values[d], %						
ME, Mcal/kg	2.95	3.07	3.21	3.08	3.13	3.27
NE, Mcal/kg	2.36	2.44	2.56	2.46[f]	2.49	2.59
Crude protein	16.94	15.66	14.89	14.70	12.83	12.10
Total lLysine	0.81	0.90	0.99	0.65	0.70	0.76
SID[e] lysine	0.64	0.77	0.91	0.49	0.58	0.67
SID lysine:ME ratio, g/Mcal	2.3	2.6	2.8	1.7	1.9	2.1
Diet free lysine equivalent[g]	0	0.16	0.30	0	0.14	0.22
SID threonine	0.48	0.49	0.59	0.39	0.39	0.45
SID isoleucine	0.27	0.27	0.33	0.14	0.14	0.20
SID lysine	0.64	0.77	0.91	0.49	0.58	0.67
SID methionine	0.25	0.24	0.32	0.22	0.20	0.22
SID threonine	0.48	0.49	0.59	0.39	0.39	0.45
SID tryptophan	0.16	0.15	0.16	0.13	0.11	0.12
ME, Mcal/kg	2.95	3.07	3.21	3.08	3.13	3.27
SID lysine:ME ratio, g/Mcal	2.29	2.57	2.79	1.69	1.94	2.08
Linoleic acid	1.30	1.48	1.68	1.50	1.57	1.76
Chemically determined values, %						
Crude protein	16	13	13	16	14	12
Total Lysine	0.86	1.04	0.99	0.69	0.67	0.74
Free Lysine	0.02	0.14	0.20	0.03	0.13	0.20
Methionine	0.24	0.20	0.19	0.25	0.20	0.20
Threonine	0.75	0.48	0.49	0.77	0.59	0.50
Tryptophan	0.18	0.16	0.18	0.20	0.18	0.17

[a] Maternal line = Large White × Landrace.
[b] Premix provided the following minerals per kg: Mn, 19 mg; Zn, 77 mg; Fe, 77 mg; Cu, 12 mg; Se, 171 ppm; I, 400 ppm; Cr, 114 ppm.
[c] Premix provided the following vitamins per kg: vitamin A, 20,566,783 IU; vitamin D3, 2,932,099 IU; vitamin E, 117,504 IU; vitamin B12, 73 mg; Biotin, 589 mg; Menadione, 9700 mg; Riboflavin, 14,698 mg; d-Pantothenic acid, 58,790 mg; niacin, 88,183 mg; folic acid, 4409 mg.
[d] Calculated using ME values for the ingredients obtained from the NRC (2012).
[e] Standard Ileal Digestible; calculated using SID coefficients for the various ingredients obtained from the NRC (2012).
[f] Energy value of this diet elevated because more fat was required to provide a minimum of 1.50% linoleic acid; one of two parent essential fatty acids.
[g] Free lysine is computed from the ingredient liquid L-lysine which is 50% lysine by multiplying the diet percent x 0.50; This can be compared to the analyzed free lysine in the diet; The latter accounts only for added synthetic lysine, not which is ingredient bound as protein.

growth rates and/or altered body composition. Reduction in growth rates of developing gilts has been reported to be associated with improved productivity (Klindt et al., 2001). Successful development of diets would be used in a larger experiment to determine their possible effect on longer term reproductive performance. Previous studies suggested that gilt growth rate and body composition could be manipulated by altering amino acid density (Rozeboom, 2007), energy intake (De Greef, 1992) and/or by restricting the daily amount of feed offered per gilt (Baidoo, 2001). In a previous study, however, growth rate and body composition of gilts did not differ when fed diets containing either a control concentration of SID lysine in grower (1.02%) and finisher (0.85%) diets or a diet with less SID lysine in grower (0.86%) and finisher (0.73%) diets (Calderón Díaz et al., 2015a).

The initial study was conducted with six diets fed *ad libitum* that contained two SID lysine and three metabolizable energy (ME) concentrations that bracketed those currently used by commercial swine herds in the USA. Growth rates, body composition and age at puberty did not differ (Calderón Díaz et al., 2015a, 2015b). Results indicated that gilts compensated for differences in ME between diets by altering feed intake. There were also no differences in response to SID lysine concentrations. Experimental SID lysine concentrations were based on an informal survey of producers and were greater than the SID lysine estimated requirements recommended by the National Research Council (NRC, 2012) and the National Swine Nutrition Guide (Whitney and Masker, 2010). Thus, the results indicated that even the least SID lysine concentration used was sufficient to maximize growth and development. The effect of lesser concentrations of SID lysine in grower and finisher diets on growth rate, body composition and age at puberty have not been evaluated. Therefore, rather than alter ME, the objective of the present study was to assess the effect of deficient, adequate and excess dietary lysine on growth, body composition and age at puberty.

2. Materials and methods

2.1. Care and use of animals

This study was approved by the Institutional Animal Care and Use Committee of the U.S. Meat Animal Research Center (Clay Center, Nebraska), and was conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching as issued by the American Federation of Animal Science Societies (FASS, 2010).

Maternal line crossbred Large White × Landrace gilts ($n = 641$) were used in this study. The study was conducted at Smithfield Hog Production facilities near Milford, Utah. Gilts for this experiment originated from sows from parities 1 through 6. Gilts (n = 72) were moved on a weekly basis for 9 weeks to the gilt development unit at 77 days of age. Within each weekly group, 24 gilts were randomly allotted to each of three pens (one pen per diet) such that littermates and gilts originating from the same parity sows were distributed evenly among pens. Pens (2.9 m × 7.2 m, minimum space per gilt = 0.93 m^2) used in the study had fully slatted concrete (slat width = 15.2 cm; space between slats = 2.5 cm) flooring in which a feeder with five feeding spaces (30.5 cm each) was centrally positioned.

Each dietary treatment consisted of a grower and finisher phase (Table 1). Gilts were fed a grower diet *ad libitum* from 100 until 142 days of age. Finisher diets were fed from 143 days of age until 220 days of age. To design diets that were adequate, deficient or excessive in amino acid to energy content (g SID lysine:Mcal ME ratio), recent estimates were used of the requirement (100% asymptote) that was recently determined for the modern lean pig (Zier-Rush et al., 2013). The estimate of lysine (lysine:metabolizable energy) adequacy was used as the adequate concentration, with deviations of greater or lesser concentrations representing excess and deficit diets for respective phases. Diets were not made isocaloric by oil addition so that any differences that resulted from dietary treatments were due to growth rate and not body composition. Grower diets were formulated to provide 0.68% (2.29 g/Mcal, low), 0.79% (2.57 g/Mcal, medium), or 0.90% SID lysine (2.79 g/Mcal, high), with a respective increase in ME (metabolizable energy) from 3.14 to 3.28 Mcal ME/kg diet. Gilts were provided corresponding finisher diets *ad libitum* that contained 0.52% (1.69 g/Mcal, low), 0.60% (1.94 g/Mcal, medium) or 0.68% (2.08 g/Mcal, high; Table 1) SID lysine from 143 days to 220 days of age, with a respective increase in ME from 3.19 to 3.28 Mcal/kg. To prevent possible compensatory feed intake in response to reduced SID lysine, and to help limit intake generally, corn germ was added to the medium and low SID lysine diets to increase dietary Neutral detergent fiber concentration (NDF). Corn germ was selected because it is commercially available and has a presumed high fraction of indigestible NDF. Total NDF was set to 9.9%, 15.6% and 19.5% for low, medium and high SID lysine diets for the grower phase and to 10.1%, 15.6% and 21.0% for respective treatments in the finisher phase. The diet model used a low digestible NDF ingredient to limit increased dietary intake adjustments, as is known to occur in the ruminant (Oba and Allen, 1999). The formulated total and SID lysine content of the diets were estimated by multiplying the total amount of each ingredient by the SID value for each ingredient obtained from the NRC (2012) and summing the values. Diet samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories (University of Missouri at Columbia, MO, USA) for proximate and lysine analysis to verify concentrations in the diets. The finisher diet was subsequently specified to contain not less than 1.50% of the essential fatty acid, linoleic acid, in an attempt to provide a minimum input as a precaution for reproduction function; even though a recommended concentration has not been established in pigs (NRC, 2012).

2.2. Measurements

2.2.1. Body composition

Gilts were individually weighed using a digital scale (Digi-Star SW4600EID Digital RFID, VID Recording scale; Digi-Star LLC, Fort Atkinson, WI, USA) and BF and LD were measured at the 10th rib using real time ultrasonography. Images were obtained by a trained technician using an EXAGO ultrasonograph (S.E.C. Repro inc., Québec, Canada) and stored and interpreted using the Biosoft Toolbox

II for Swine (Biotronics Inc, Ames, IA, USA) at 100 days of age and then every 4 weeks until 212 days of age (last measuring date). A fat-to-lean ratio was calculated by dividing BF by LD.

2.2.2. Feed intake

Feed intake (FI) was recorded as feed disappearance per pen every 2 weeks. Lysine (g) consumed every 2 weeks was calculated by multiplying the formulated dietary lysine by the feed consumed (kg). Average daily feed and lysine intake per pig were calculated by dividing the total FI and lysine intake by the number of pig days (*i.e.* number of pigs in the pen multiplied by the number of days each pig remained in the pen) per pen, respectively. Average daily gain and other intake variables were calculated for each 28 day interval. Feed and lysine intake per kg of body weight (BW) gain were also calculated for each 28 day interval.

2.2.3. Age at puberty

At 160 days of age, gilts were exposed daily to a rotation of mature (> 10 months of age) vasectomized boars using direct single boar contact each day for 10 min. Individual gilt behavior and changes in vulval condition were recorded daily as previously described (Calderón Díaz et al., 2015b). Briefly, scoring was as follows: 0 = no signs of pro-estrus/estrus (gilt shows no interest towards boar and no vulval changes); 1 = gilt "soliciting" the boar (head to head contact) or with a reddened vulva; 2 = gilt allowing head-to-flank by boar or displaying a swollen, reddened vulva with vulval discharge; 3 = standing estrus when applying the back pressure test in the presence of the boar. The first day of standing estrus (score 3) was considered pubertal estrus. On the day that standing estrus was observed, a flank to flank measure, BW, BF and LD were measured. Gilts were observed daily for signs of standing estrus until 220 days of age. Blood samples were collected from all gilts that had not been observed in standing estrus at 210 and 220 days of age for progesterone analysis, to evaluate whether each gilt that failed to reach puberty was either prepubertal or behaviorally anestrus. At 220 days of age, all gilts that had not had a pubertal estrus were injected with PG-600 (5 ml) to induce puberty. Following PG-600, gilts were observed for estrous behavior for an additional week to determine their response.

Blood samples were allowed to clot, centrifuged to collect serum, and serum progesterone was measured by radioimmunoassay (RIA). Progesterone concentrations were estimated with a solid-phase RIA (ImmuChem Coated Tube RIA kit, MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's recommendations with the following modifications: 100 µl of phosphate buffered saline (0.01 M) were included and the incubation time was lengthened to 3 h. Using these assay conditions, porcine serum with progesterone measuring 26.1 ng/mL was serially diluted up to 32 fold and measured by RIA. Measured concentrations in the serially diluted samples were parallel to the standard curve. When serum pools from ovariectomized and prepubertal gilts were included, concentrations of progesterone were below the sensitivity of the assay (0.15 ng/mL). A porcine serum pool measuring 27.5 ng/mL was included in each assay. Intra- and inter-assay CV were 8.6% and 15.7%, respectively. Concentrations of ≥ 1 ng/mL were defined as indicative of luteal activity, which was then used to distinguish between prepubertal gilts and behaviorally anestrus gilts (*i.e.* estrous cycling but no estrous behavior).

2.2.4. PEDV outbreak

All gilts in the study contracted Porcine Epidemic Diarrhea (PED) within a 1 week period corresponding to the time when the gilts in the first week of the study were to begin boar exposure and estrous detection. Consequently, the age of gilts at the time of PED infection varied from 100 to 159 days of age, according to the weekly groupings. Boar exposure in the first weekly group was delayed 1 week to allow the gilts to recover. Boar exposure was initiated for all other groups at 160 days of age.

2.2.5. Gilts removed from study

Gilt removal from the study (e.g., sick animals, culling and deaths) was based on decisions made by the manager of the gilt development facility and were retrospectively acquired from farm records.

2.3. Statistical analysis

Pen was considered the experimental unit. Predicted variables were evaluated for normality using the Shapiro-Wilk test and examining the normal plot. Data were analyzed using mixed model equation methods (SAS v9.3 PROC MIXED; SAS Inst. Inc., Cary, NC). Initial models for BW, body composition, feed intake and feed efficiency included lysine concentration, data recording day, and their interactions with week of the study as fixed effects. The BW at the beginning of the study (*i.e.*, 100 days of age) was used as a linear covariate in the models. Age at PEDV infection (range from 100 to 159 days of age) was included as a linear covariate for growth and body composition variables. Two analyses were performed for age at puberty. The first analysis included all the gilts that were on study at 160 days of age. For the second analysis, gilts that were removed from the experiment before 220 days of age were excluded as some of them could have been removed before they reached the physiological maturity required to show standing estrus. Initial models for age at puberty included lysine concentration as fixed effect and BF, BW, LD at puberty and age at PEDV infection were included in the model as linear covariates. Pen within lysine × week was included as a random effect for all the variables analyzed. A backward stepwise selection for fixed effects was used and only fixed effects with a $P < 0.10$ remained in the final model except for lysine concentration, which was included in the model irrespective of its P value. Statistical differences were reported when model source of variation was $P \leq 0.05$. When a main effect was a significant source of variation, concentrations from each main effect were separated using the PDIFF option and a Tukey–Kramer adjustment was used to account for multiple comparisons between concentrations. Results for fixed effects are reported as least squares means $\pm$ standard error (SE). Results for continuous variables are reported as the regression coefficient (REG) $\pm$ SE.

Incidence of naturally occurring puberty, incidence of response to PG-600 injection and reasons for removing gilts from the study were analyzed using a Chi Square test (SAS v9.3 PROC FREQ; SAS Inst. Inc., Cary, NC). A univariate binomial logistic regression model (SAS v9.3 PROC GENMOD; SAS Inst. Inc., Cary, NC) was fitted to evaluate the effect of age at PEDV on the likelihood of showing standing estrus after boar stimulation started. The linear relationship between flank to flank measures and weights at natural estrus was generated using excel.

3. Results

Seventy-six gilts (11.8%) were removed from the study. Thirty-four gilts died, four gilts were euthanized and 38 gilts were removed for various reasons. Poor body condition was the most common cause for removal (60.5%), 18.4% were removed due to lameness and 21% of gilts did not have a record of a reason for removal. There was, however, no difference among lysine concentration treatments in the total number of gilts removed ($P > 0.05$).

3.1. Analysis of diets

Chemical analyses of the grower and finisher diets in this experiment are summarized in Table 1. Amino acid analysis indicated that total Lysine in each diet was within 6% of that expected, except for the Medium Grower diet, which differed from that predicted by 16%.

3.2. Growth and body composition

Table 2 shows the descriptive statistics for the measurements recorded at initiation of the diets at 100 days of age. Gilts fed the low lysine diet had lesser average daily gain (ADG; Table 3) and BW when compared to gilts feed the medium and high lysine diets ($P < 0.0001$). Likewise, gilts fed the medium lysine diet had lesser BW when compared to gilts fed the high lysine diet ($P < 0.0001$; Table 3). There was no difference between gilts fed the low and medium lysine diets for BF and LD ($P > 0.05$). With both dietary treatments, however, BF and LD were less when compared to gilts fed the high lysine diet ($P < 0.05$). Even though there were differences in BW, BF and LD, no differences were observed in the fat-to-lean ratio among dietary treatments ($P = 0.3741$). As expected, growth increased and body composition changed with age ($P < 0.0001$; Table 3). Gilts with greater BW at 100 days of age had greater values for BW, BF thickness and LD throughout the study ($P < 0.0001$). Age at PEDV infection was not a significant source of variation for any of the body composition variables ($P > 0.05$).

3.3. Feed intake and feed efficiency variables

Gilts fed the low lysine diet had a greater average daily FI and less average daily lysine intake when compared to gilts fed the high lysine diet ($P < 0.05$), but did not differ from gilts fed the medium lysine diet ($P > 0.05$; Table 3). Additionally, average daily feed and lysine intakes were similar between gilts fed the medium and high lysine diets ($P > 0.05$). Average daily feed and lysine intakes were related to BW at 100 days of age ($P < 0.0001$). Additionally, gilts fed the low lysine diet had a greater FI per kg of BW gain compared with gilts fed the high lysine diet ($P < 0.05$), but there were no observed differences in the grams of lysine required per kg of BW gain among dietary treatments ($P > 0.05$; Table 3).

3.4. Age at puberty

Average age at puberty was 202 days of age with a range from 166 to 222 days of age. Fifty-four, 62 and 77 gilts representing 27.7%, 31.0% and 37.7% from the low, medium and high lysine dietary treatments attained puberty and there were no statistical differences among dietary treatments ($P = 0.12$). When all gilts that were on study at 160 d of age were included in the data analysis,

Table 2
Descriptive statistics for body weight, backfat thickness, loin depth and fat-to-lean ratio of maternal line[a] gilts fed *ad libitum* diets three lysine concentrations beginning at 100 days of age.

	Low lysine[b]				Medium lysine[c]				High lysine[d]			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Body weight (kg)	46	7.8	25	64	46	6.8	30	62	46	7.2	25	67
Backfat thickness (mm)	7.2	1.5	4.0	14	7.3	1.4	3.3	12	7.2	1.6	4.0	13
Loin depth (cm)	3.8	0.56	2.2	5.0	3.7	0.51	2.4	5.0	3.7	0.58	2.3	5.4
Fat-to-lean ratio	0.19	0.03	0.11	0.33	0.20	0.03	0.13	0.31	0.19	0.04	0.11	0.34

[a] Maternal line = Large White × Landrace.
[b] Grower diet: 0.68% SID lysine; Finisher diet: 0.52% SID lysine.
[c] Grower diet: 0.79% SID lysine; Finisher diet: 0.60% SID lysine.
[d] Grower diet: 0.90% SID lysine; Finisher diet: 0.68% SID lysine.

Table 3

Least square means ± SEM for growth, feed intake variables and body composition variables for different periods between 128 and 212 days of age of maternal line[1] gilts fed *ad libitum* diets with three lysine concentrations from 100 days of age.

	Low lysine[2]		Medium lysine[3]		High lysine[4]		BW at 100 days of age[5]
	LS mean	SEM	LS mean	SEM	LS mean	SEM	
Body weight (kg)	***						
128 d	62[†,a]	0.69	65[‡,a]	0.69	69[§,a]	0.69	1.52(0.055)***
156 d	80[†,b]	0.70	86[‡,b]	0.69	91[§,b]	0.69	
184 d	104[†,c]	0.71	111[‡,c]	0.70	120[§,c]	0.70	
212 d	130[†,d]	0.72	138[‡,d]	0.72	148[§,d]	0.72	
Average daily gain (kg)	*						
128 d	0.54[†,a]	0.04	0.66[‡,a]	0.04	0.78[§,a]	0.04	NI[6]
156 d	0.63[†,a]	0.04	0.71[‡,a]	0.04	0.79[§,a]	0.04	
184 d	0.83[†,b]	0.04	0.88[‡,b]	0.04	0.99[§,b]	0.04	
212 d	0.88[†,b]	0.04	0.90[‡,b]	0.04	0.93[§,b]	0.04	
Average daily feed intake (kg)	ns[7]						
128 d	2.5[†,a]	0.15	2.5[†,a]	0.14	2.5[†,1]	0.14	0.022(0.002)**
156 d	2.7[†,a]	0.12	2.6[†,‡,a]	0.11	2.4[‡,1]	0.11	
184 d	2.6[†,a]	0.10	2.4[†,‡,a]	0.11	2.3[‡,1]	0.12	
212 d	2.6[†,a]	0.13	2.4[†,‡,a]	0.15	2.4[‡,1]	0.17	
Feed intake per kg of BW gain (kg)	ns						
128 d	3.1[†,a]	0.20	2.7[‡,a]	0.20	2.3[§,a]	0.20	NI
156 d	3.9[†,a]	0.20	3.3[‡,b]	0.20	2.8[§,a]	0.20	
184 d	3.2[†,a]	0.20	3.0[‡,1,b]	0.20	2.8[§,b]	0.20	
212 d	3.7[†,b]	0.20	3.6[†,b]	0.20	3.7[†,a,b]	0.20	
Average daily lysine intake (g)	ns						
128 d	17[†,a]	0.99	19[†,‡,a]	0.95	20[‡,a]	0.90	0.15(0.019)**
156 d	17[†,a]	0.77	18[†,‡,a]	0.72	19[‡,a]	0.68	
184 d	13[†,b]	0.66	14[†,‡,b]	0.69	16[‡,b]	0.76	
212 d	12[†,b]	0.87	14[†,b]	0.98	16[‡,b]	1.1	
Lysine intake per kg of BW gain (g)	ns						
128 d	21[†,a]	1.3	21[†,a]	1.3	20[†,a]	1.3	NI
156 d	23[†,a]	1.3	23[†,a]	1.3	22[†,a]	1.3	
184 d	17[†,b]	1.3	18[†,b]	1.3	19[†,b]	1.3	
212 d	19[†,b]	1.3	22[†,b]	1.3	25[†,a]	1.3	
Backfat thickness (mm)	***						
128 d	7.0[†,a]	0.19	7.6[†,‡,a]	0.19	8.1[‡,a]	0.19	0.15(0.015)***
156 d	8.9[†,b]	0.19	9.7[†,b]	0.19	10[‡,b]	0.19	
184 d	12[†,c]	0.20	13[†,c]	0.19	15[‡,c]	0.19	
212 d	16[†,d]	0.20	17[†,d]	0.20	19[‡,d]	0.20	
Loin depth (cm)	***						
128 d	3.8[†,a]	0.03	4.1[‡,a]	0.03	4.4[§,a]	0.03	0.045(0.003)***
156 d	4.2[†,b]	0.03	4.7[‡,b]	0.03	5.0[§,b]	0.03	
184 d	4.9[†,c]	0.04	5.4[‡,c]	0.03	5.8[§,c]	0.03	
212 d	5.6[†,d]	0.04	6.2[‡,d]	0.04	6.6[§,d]	0.04	
Fat to lean ratio	*						
128 d	1.9[†,a]	0.03	1.8[†,a]	0.03	1.8[†,a]	0.03	0.009(0.002)**
156 d	2.1[†,b]	0.03	2.1[†,b]	0.03	2.1[†,b]	0.03	
184 d	2.5[†,c]	0.03	2.4[†,c]	0.03	2.6[†,c]	0.03	
212 d	2.9[†,d]	0.03	2.7[†,d]	0.03	2.9[†,d]	0.03	

[†,‡,§]Within rows, significant differences between predictor variables lysine.

[a,b,c,d]Within columns, Significant difference between periods.

[1] Maternal line = Large White × Landrace.

[2] Grower diet: 0.68% SID lysine; Finisher diet: 0.52% SID lysine.

[3] Grower diet: 0.79% SID lysine; Finisher diet: 0.60% SID lysine.

[4] Grower diet: 0.90% SID lysine; Finisher diet: 0.68% SID lysine.

[5] Results for continuous variables presented as the regression coefficient ± SE.

[6] ns = non-significant effect of lysine concentration by period; $P > 0.05$.

[7] NI = not included in the model.

*** Significant effect of the interaction between predictor variable lysine concentrations by period; $P < 0.0001$.

** Significant effect of the interaction between predictor variable lysine concentrations by period; $P < 0.01$.

only 32% of gilts had a standing estrus by 220 days of age. Thirty-four gilts were removed from the study after boar stimulation started but three gilts showed standing estrus before removal. When gilts removed from the study before 220 days of age were excluded from the analysis, 34.1% of gilts displayed standing estrus. The number of gilts that reached puberty was not different

among dietary treatments when either analysis was used ($P > 0.05$). Gilts fed the high and medium lysine diets, however, reached puberty 10 and 6 days earlier than gilts fed the low lysine diet, respectively (198 ± 2 and 202 ± 2 days for high and medium diets, respectively compared with 209 ± 2 days for low diets; $P < 0.05$). Overall, gilts that reached puberty later had a greater BW at puberty (REG $= 0.45 \pm 0.05$; $P < 0.05$) compared with gilts that reached puberty early in the study. By contrast, gilts that reached puberty later had less BF (REG $= -0.45 \pm 0.22$; $P < 0.05$) and ADG at puberty (REG $= -22.89 \pm 12.11$; $P < 0.05$).

Of the gilts failing to express puberty by 220 days of age, 19 gilts (5% of gilts on study; 5, 8 and 6 gilts from the low, medium and high lysine dietary treatments, respectively) were determined to be behaviorally anestrus in that they had progesterone concentrations greater than 1 ng/mL in samples collected at either 210 or 220 days of age. Nevertheless, 47% (9 of 19) of these gilts responded to PG-600 treatment by expressing estrus within 1 week post-treatment administration. Age at PEDV was not a significant source of variation for the likelihood of naturally achieving puberty. Gilts that were infected with PEDV at an earlier age, however, tended to be less likely to express standing estrus when compared to gilts that were infected by the virus at an older age ($P = 0.09$). Number and percentage of gilts that expressed puberty by treatment in each weekly batch of gilts is presented in Table 4.

Fig. 1 depicts the relationship between BW and flank to flank measures obtained at puberty for each gilt that expressed a natural puberty in this experiment by 220 days of age. Gilts averaged 115 kg at approximately 76.2 cm flank to flank. From the graph, a threshold value of 84 cm flank to flank measurement would ensure that all gilts were at least 115 kg, however, at this threshold value, the average weight was 150 kg and 20% of gilts exceeded 160 kg at puberty.

4. Discussion

Development of diets that differed in lysine density (lysine, lysine:ME), combined with corn germ to provide NDF, was successful in providing growth limiting diets that were fed *ad libitum*. Gilts fed the low lysine (and lless ME) diets had a slightly increased dietary intake, however, they were unable to increase intake enough to avoid the amino acid deficit. This diet model could have been improved by the addition of more indigestible NDF, however, growth was significantly altered with no apparent change in the composition of growth (Table 3).

The feeding model used in the present study recognized that intake is highly regulated for energy and nutrient input (NRC Swine, 2012). Energy density is a key modifying dietary intake factor in pigs, however, amino acid dietary content that is less than that recommended by the NRC (NRC, 2012) can also be partially compensated for by increasing intake (Calderón Díaz et al., 2015a, 2015b). The diet model of the present study involved increasing diet total NDF to ultimately result in a maximum dietary intake. Not much is known about NDF use to ultimately control dietary intake in pigs, however, there is a greater understanding in ruminants (Thonney and Hogue, 2013). In ruminants, total NDF is not a very reliable predictor of intake, however, increasing the indigestible fraction of total NDF has a suppressing effect on dietary intake. Use of the ruminant procedure in the present study provided the desired outcome of delivering target SID lysine concentrations by greatly reducing animal adaptation in intake in response to energy or amino acid concentrations in the diet.

The proportion of gilts removed from the present study was similar to those previously reported (Calderón Díaz et al., 2013, 2015a; Knauer et al., 2011). Unlike previous studies where leg problems were reported as the main reason for gilt removals (Boyle et al., 1998; Engblom et al., 2007); the main cause of removal from the present study was poor body condition. Because there was no difference in the number of animals removed between dietary treatments, the large number of gilts removed due to poor body condition may have been related to the gilts being affected by PEDV.

Results from the present study suggest that BW can be successfully decreased without altering body composition by feeding diets to gilts *ad libitum* that contain reduced SID lysine concentration (Table 3). These results are consistent with the finding that, in general, calculated total lysine concentrations in the diets were similar to analyzed values for all diets (*i.e.*, within 6%), except for the medium lysine grower diet. The greater measured total lysine value (16%) for the medium lysine grower diet is a cause for some concern of results in the present study, however, the free lysine addition to this diet is consistent with calculated and analyzed values.

Table 4
Incidence of natural occurring puberty by 220 days of age in each weekly group by treatment of maternal line[1] gilts fed diets *ad libitum* with three lysine concentrations from 100 days of age.

Group	n	Low[a]		Medium		High	
		Number	Percentage	Number	Percentage	Number	Percentage
1	71	5	7.0	9	13	5	7.0
2	72	3	4.2	3	4.2	5	6.9
3	71	3	4.2	9	12	10	14
4	72	4	5.6	5	6.9	10	14
5	71	6	8.5	5	7.0	11	15
6	70	12	17	13	19	13	19
7	71	6	8.5	8	11	14	20
8	71	11	15	4	5.6	3	4.2
9	72	4	5.6	6	8.3	6	8.3

[1] Maternal line = Large White × Landrace.
[a] Number and percentage responders did not differ between lysine concentrations.
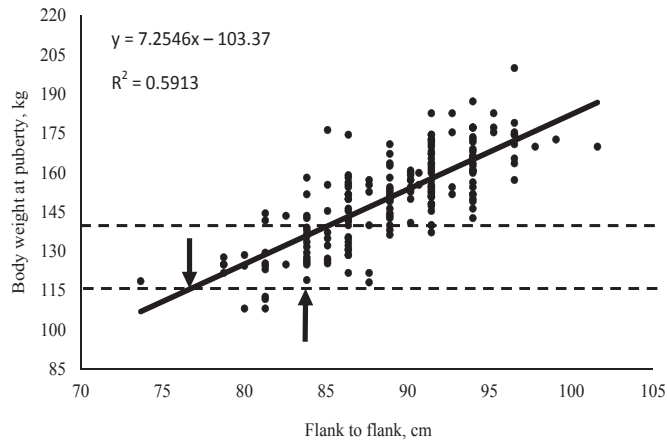
Fig. 1. Scatterplot showing the relationship between flank to flank measure and body weights obtained at puberty of maternal line crossbred Large White × Landrace gilts fed diets *ad libitum* with three lysine concentrations from 100 days of age. Regression line is indicated by the solid line. A 116 kg minimum weight threshold (lower dashed line) that assumes gilts will grow at 0.9 kg per day for an additional 21 days for a subsequent estrus cycle is indicated, to result in a breeding weight of 135 kg. A 140 kg upper weight threshold is indicated by the upper dashed line, which would result in a 160 kg weight at breeding. The first arrow from right to left indicates the flank to flank measure corresponding to a mean of approximately 115 kg, where gilts will average 135 kg at breeding. The second arrow indicates the flank to flank measure where all gilts will be above the minimum threshold of 115 kg at puberty, where all the gilts are predicted to be above 135 kg at breeding. Note the number of gilts above the upper weight threshold.

Further, it is clear from data in Table 3 that gilts fed the medium grower diet had already begun to diverge in BW and LD at 128 days of age as compared with gilts fed the high lysine grower diet. This indicates that even though the total lysine analysis suggested lysine concentrations were higher than expected, the diet had the desired effect on BW and LD.

Similar reductions in BW were reported by Cia et al. (1998) in a study where gilts were fed diets differing in lysine concentrations and feed was provided based on BW with the intent that intake would not be limited. Diets were based on DE but energy concentrations provided in the diets were similar to those of the current experiment. It was reported in this previous study that gilts fed the low (0.46% total lysine) and medium (0.92% total lysine) lysine diet had progressively less BW throughout the experimental period when compared with gilts fed the high lysine (1.38% total lysine) diets. Cia et al. (1998) also reported that BF was greater in both the medium and low lysine diet groups compared to the high lysine group, which is not consistent with the current results in which BF was similar between treatments. The different results could be due to the fact that, in the study by Cia et al. (1998), diets were fed on a multiple of maintenance requirements to minimize differences in feed refusal. The multiple was adjusted on a weekly basis as the study progressed, such that all animals in the treatment groups had similar dietary intakes. Feed consumption was reduced in the high lysine group, and the intakes in the other groups were adjusted accordingly, thus feeding was not truly *ad libitum*. In the current study, gilts were fed *ad libitum*. It may be possible that given the artificial adjustment of intake in the previous study, gilts fed the low lysine diet consumed less protein than optimal for growth of lean tissues, altering metabolism toward fat deposition. Similar to the study of Cia et al. (1998), lean tissue deposition was progressively less in the medium and low lysine groups in the current study as measured by LD. An alternative hypothesis is that a source of fiber was included in the reduced lysine diets in the current study which did not occur in the previous study. The addition of fiber in the diets may result in decreased fat deposition. This is consistent with recent reports that high fiber diets result in decreased long-term weight gain in humans (Menni et al., 2017) and high-fat-diet-induced obesity in rats (Chang et al., 2017). The present study was not specifically designed to assess the effects of fiber on fat accretion, and further studies are needed.

As the low lysine diet had slightly less dietary energy concentration, it is not surprising that gilts fed the low lysine diet had a slightly greater daily feed intake compared with gilts fed the high lysine diet as gilts adapt to dietary differences by changing intake due to the dietary energy concentration (Calderón Díaz et al., 2015a; Tokach et al., 2000). There was greater feed intake in the present study even though there was the addition of corn germ, which was included in the diets to inhibit compensatory feed intake. This suggests that concentrations of indigestible NDF were not great enough in the present study to fully equate the intake of the diets. Even with the greater feed intake, however, gilts fed the low lysine diet still had less daily lysine intake. This is due to the fact that the difference in feed intake (between the low and high lysine diet) was not great and thus, the gilts did not fully compensate for the reduced lysine concentration in the low lysine dietary treatment. Nonetheless, average daily lysine consumption in the present study was within the range for optimal daily lysine intake reported in other studies (Sørensen et al., 1993; Friesen et al., 1995; Hahn et al., 1995) even for the gilts fed the diet where the least SID lysine concentration was provided. Furthermore, irrespective of dietary treatments, gilts consumed 20 g of lysine/kg BW gain in the present study which has been reported as the optimal amount of lysine needed per kg of BW gain (De La Llata et al., 2007; Main et al., 2008; Shelton et al., 2009).

Gilts fed the diets with high and medium lysine concentrations in the present study expressed puberty earlier than gilts fed the low lysine diet. This is likely related to growth rate as gilts that were fed the high lysine diet also had a greater ADG at puberty and throughout the study. This result is in agreement with those previously reported where gilts with a greater growth rate expressed puberty at an earlier age (Hutchens et al., 1981; Rydhmer et al., 1992; Kummer et al., 2009). Although it has been reported that

feeding gilts diets with low lysine concentrations does not impair puberty attainment (Levis et al., 1997), only 31.1% of gilts had a standing estrus in the present study after boar stimulation started. As the number of gilts that expressed standing estrus did not differ between treatments, it is likely that the small number of gilts that reached puberty was due to non-nutritional factors (e.g., PEDV). Studies have suggested that a minimum BW (*i.e.*, 125 kg; Yang et al., 1989), BF thickness (*i.e.*, 16 mm; Tarres et al., 2006) and/or growth rate (*i.e.*, 0.6 kg/day; Foxcroft and Aherne, 2001) is necessary to attain puberty. None of these thresholds would, however, explain the small number of gilts that expressed standing estrus as gilts in the present study had similar or greater values for growth rate as those in previous studies.

Although not statistically significant, gilts that were infected with PEDV at an earlier age tended to be less likely to express standing estrus than gilts that were infected by the virus at an older age in the present study which is consistent with an earlier report where severity of PEDV decreases with age (Pensaert and Yeo, 2006). It is possible that if gilts had remained in the present study for a longer period of time, they may have had enough time to recover from the PEDV effects on their immune system and the pubertal estrus would have been observed in a greater number of gilts as compared with what occurred. It was not possible, however, to continue estrous observations for a longer period of time as the present study was conducted in a commercial farm and common commercial practices for estrous detection were followed. Indeed, most gilts that did not express estrus by 220 days of age were still pre-pubertal rather than in behavioral anestrus, as only 5% had detectable progesterone concentrations in plasma at 210 and 220 days of age and 87.8% of gilts treated with PG-600 responded with a standing estrus within 1 week of the time of this treatment. Interestingly, nearly half of gilts were behaviorally anestrus, however, ovulations had occurred as determined by progesterone analysis in the present study and were, therefore, estrous cycling and these gilts responded to the PG-600 treatment by expressing behavioral estrus. These gilts were likely to have been at the stage of the estrous cycle that did not preclude them from responding to PG-600, and the PG-600 stimulation likely facilitated the expression of estrous behavior during the subsequent period of follicular growth and ovulation. This suggests that PG-600 treatment of behaviorally anestrus gilts may provide some benefit in terms of stimulating expression of estrus, although it is not clear whether these gilts would express estrus at the subsequent estrous cycle for successful mating.

Although gilts fed the low lysine diet had a lesser average daily gain, all gilts in the present study had lifetime growth rates greater than 0.6 kg/day which has been suggested as a minimum daily growth rate for puberty attainment (Foxcroft et al., 2005). Beyond this growth rate threshold, age at puberty is influenced less by body weight, which is consistent with the positive relationship between age at puberty and weight in the present experiment. If puberty occurred at a threshold weight, there would be no relationship between puberty age and weight.

Weights and flank to flank measures taken at estrus in the present experiment provide an opportunity to examine relationships between weights, flank to flank measures, puberty and recommended weights at breeding, which would typically take place at second or later estrus relative to time of puberty. Recommendations in the literature indicate that gilts should be bred when they are at a weight greater than the threshold of 135 kg but before they reach 160 kg (Bortolozzo et al., 2009). Given growth rates of approximately 0.9 kg per day (Table 3) and assuming a 21 day estrous cycle before breeding, the minimum weight threshold at puberty should be 116 kg, which would correspond to 76.2 cm flank to flank for an average weight of 116 kg, or 84 cm if all gilts are at the minimum weight criterion. A similar upper threshold can be calculated using the same strategy such that gilts should not exceed 140 kg at first estrus.

Weighing gilts is labor intensive, and correlated measures have been developed that do not require moving gilts to a scale (Iwasawa et al., 2004; Sungirai et al., 2014). Flank to flank measurement is correlated with weight as previously reported, however, if 84 cm is used to ensure that all gilts are at or greater than the weight threshold, 80% of gilts that did not meet the flank to flank threshold were actually at a weight greater than the minimum threshold. Even with the reduced growth rates caused by the medium and low lysine diets, all but four gilts were at the 116 kg weight criterion at puberty. If the 76 cm threshold is used such that gilts average 116 kg, only one gilt was below this threshold, and her weight actually exceeded the 116 kg threshold. In addition, four gilts had a weight that was less than the threshold even though they met the flank to flank threshold.

Results also indicate that even with the low lysine concentration diet in the present experiment, most gilts were likely to reach the minimum weight threshold at breeding. However, 72% of gilts in this experiment weighed greater than 140 kg at puberty, and so were predicted to exceed 160 kg at breeding. Exceeding the upper threshold has been reported to be associated with structural problems (e.g., lameness) and failure to return to estrus after weaning during later parities (Bortolozzo et al., 2009). If excessive weights are detrimental, the current results indicate that gilts that are developed by feeding the medium and low lysine diets may have a subsequent advantage in terms of herd retention. The average weight at the first estrus of gilts that reached puberty spontaneously that were fed the low lysine diet was 144 kg, close to the upper threshold. For gilts in which estrus was induced, the average weight at induced puberty was similar (143 kg). This compares to 157 kg for natural puberty and 159 kg for PG600 induced puberty for gilts fed the high lysine diet. Thus, the low lysine diet could be useful in limiting the number of gilts that exceed a recommended weight threshold at first breeding, should it be supported by further research. The diets developed in the present experiment will be used in a subsequent larger study to examine litter variables at three parities. This will allow for confirmation as to whether there is an upper weight threshold for breeding that is warranted, and whether a low lysine diet provides an advantage by reducing the incidence of excessive weight at breeding.

In conclusion, under the conditions of the present study, results indicated that growth of developing gilts can be altered when feeding diets *ad libitum* that were differing in SID lysine concentration without altering body composition. Even gilts fed the low SID lysine diet had a growth rate, BW and body composition that was greater than the threshold for puberty attainment, although age at puberty was delayed by 6–10 days. This suggests that growth rates can be reduced without major effects on puberty onset. Results from the present study regarding number of animals that expressed standing estrus need to be treated with caution, however, as the

farm experienced an outbreak of PEDV, which could have negatively impacted gilt puberty attainment. It is not believed, however, that contraction of this disease affected treatments differentially. Nevertheless, these results indicate that reduction in g dietary SID lysine intake per kg BW could be useful in reducing weights at first mating, which has been reported to be a factor affecting retention in the breeding herd. This concept will be further investigated in a future long term study where the breeding herd productivity of a large number of gilts will be followed during three parities.

Conflict of interests

The authors have no conflicts of interest with regard to these results.

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