RESEARCH REPORT
ANIMAL SCIENCE

Title: Optimal dietary protein for the development of gilts. **NPB #15-119 (14-235-Year2)**

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Industry Summary:

This experiment was undertaken to develop diets that could be fed ad libitum to developing gilts that would result in differences in growth rate or body composition during development. A further component of the experiment was to determine whether diets affected age at puberty. This study is a prelude to a larger study to measure dietary affects on sow productivity to third parity. Diets were developed that had normal levels of metabolizable energy (~3200 kcal/kg) but differed in SID lysine levels, with a control designed to be adequate, and two further diets with progressively less SID lysine (medium and low lysine, respectively). Diets were fed in two phases, a grower phase that was applied for 6 weeks, and a finisher phase that was fed from then until gilts left the experiment at 220 days of age. Diets were begun at 100 days of age. Gilts were weighed and measured for backfat and loin depth by ultrasound when diets began and at 28 day intervals until 212 days of age. Blood samples were collected from gilts that had not expressed estrus by 210 and 220 days of age for progesterone analysis to assess whether they were prepubertal or behaviorally anestrus. Finally, at 220 days of age all gilts not experiencing a standing estrus were injected with PG600 and observed for 1 week to determine their estrus response.

Gilts on this trial experienced a porcine epidemic diarrhea virus outbreak at approximately the time estrus detection and boar exposure was begun. It is likely that this outbreak temporarily impaired the growth of gilts, but there was no uninfected control to allow conclusions to be drawn on the PED effects, if any. On the other hand, all treatments were similarly afflicted, thus conclusions from the trial regarding comparisons among diets are likely to be valid.

Compared to the Control diet, the medium and low SID lysine diets progressively reduced body weight, loin depth and backfat gain in gilts, with no changes in relationships (body composition) between loin depth and backfat. Only approximately 30% of gilts reached puberty by 220 days in this experiment, likely due to the effects of PED. The number of gilts experiencing puberty per the number of gilts exposed to the boar was

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numerically less for the medium and low lysine diets, but these differences were not statistically significant. However, mean age at puberty was significantly greater in gilts fed the low lysine diet. Most noncyclic gilts experienced puberty in response to PG600, suggesting that they were prepubertal, rather than behaviorally anestrus. However, about half the gilts that were confirmed to be behaviorally anestrus using progesterone concentrations responded to PG600 by exhibiting standing estrus within one week.

These results indicate that we were successful in developing ad libitum fed diets that resulted in reduced growth of developing gilts by reducing the lysine level of the diet. Although the number of gilts reaching puberty did not differ, age at puberty in the low lysine diet was 7 days older than the control and medium lysine diets. These diets can now be used to determine effects of reduced growth rates on retention of gilts in the breeding herd.

Keywords: Puberty, lysine, PG600, loin depth, back fat, growth

Scientific Abstract:

A previous trial funded by the National Pork Board indicated that within the practical range of commercially available ingredients for swine diets, metabolizable energy (ME) content in the diet could not be used to alter the growth rate of developing gilts, and had only minor effects on fat deposition. In addition, a 15% reduction of SID lysine levels commonly used in industry diets to develop gilts failed to reduce growth rates. In the current trial, diets were developed that had similar ME levels (3135-3278 Mcal/kg), and different SID lysine levels of 0.90, 0.79 and 0.68% (grower diets) and 0.68, 0.60 and 0.52% (finisher diets) for control, medium and low lysine levels. Grower diets were fed beginning at 100 days of age and continued for 6 weeks, followed by finisher diets until 220 days of age. At commencement of diets and at 28 day intervals until the end of the trial, gilts were weighed and measured for back fat and loin depth using ultrasound. Gilts were exposed to mature boars beginning at 160 days of age and observed for estrous behavior once a day. At 210 and 220 days of age, blood samples were collected from all gilts that failed to reach puberty, as indicated by standing estrus. At 220 days of age, gilts received an injection of PG600 to stimulate the onset of puberty, and gilts were observed for estrous behavior for an additional week. Results indicated that growth of body weight, loin depth and back fat were progressively reduced as SID lysine in the diets were reduced, indicating that diets reduced growth rate but did not alter body composition. Age at puberty was significantly greater in gilts on the low lysine diets compared to control and medium lysine diets (209±2 days versus 202±2 and 198±2 days, respectively). The percentage of gilts reaching puberty before 220 days of age was low but did not differ among treatments (38, 31 and 28% for control, medium and low lysine, respectively). A majority of gilts that had not reached puberty experienced puberty in response to PG600 injection, and this percentage did not differ between diets (85%, 76% and 79% for control, medium and low lysine diets, respectively). The gilts in this experiment experienced a porcine epidemic diarrhea virus outbreak in unison, but varied in age at the time of the outbreak (93 to 160 days of age) because they entered the trial on a weekly basis over a 9 week period. This may explain the low rate of puberty attainment. In conclusion, these results indicate that holding ME constant and decreasing SID lysine in diets was able to delay growth of gilts without altering body composition. The reduced growth rate increased age at puberty in the low lysine diet, but did not alter the percentage of gilts reaching puberty by 220 days of age. These diets will be useful in determining the effects of reduced growth rate in ad libidum fed gilts on sow productivity in the breeding herd.

Introduction:

This is a follow on study of the effect of dietary energy and protein level on gilt development. The primary objective of the first gilt trial was to determine three diets for use in a subsequent NPB trial of dietary effects on gilt development and retention of sows in the breeding herd to fourth parity. Selection of diets was to be based on the effects of energy and amino acid levels on growth, cyclicity, and reproductive tract and mammary gland development in gilts. A second objective was to retain tissues from all animals for genomic

analyses, both for discovery of new potential markers for gilt traits and for validation of previously discovered genetic markers for gilt traits. A third objective was to look at the effects of litter of origin on gilt development, including the effects of litter size on future cyclicity and the effects of colostrum availability (as measured by the immunocrit) on adult gilt cyclicity. Results indicated that the dietary treatments failed to alter growth and cyclicity of the gilts, and caused only minor differences in the fat to lean ratio. Thus, diets for the sow trial could not be chosen with confidence, and a further gilt trial was needed (this trial) in order to test the effects of diets that were even more divergent in amino acid levels to determine their effects on growth and cyclicity. Gilts in the first trial adjusted their feed intake in response to the level of metabolizable energy in the diet, making further manipulations of dietary energy level pointless. Similar changes in intake did not occur in response to changes in lysine level, suggesting that further reductions in lysine level might meet the original objectives of the trial if the lysine was low enough.

This proposal is a second trial to accomplish the first objective of a preliminary trial proposed by the NPB. Results will be used to inform a subsequent trial that will occur as a follow on to this experiment. The two experiments together will provide results indicating the effects of lysine levels on gilt development and retention of sows in the breeding herd with sufficient animal numbers and in a commercial setting to provide results that are relevant to commercial swine production. If diets are successful in altering growth rate and fat to lean ratio in this trial, it is possible that they will improve sow retention in the breeding herd, which would be extremely valuable to commercial swine production

Objectives:

The primary objective of this project remains the same as the first objective in the initial gilt trial: determine three diets that alter growth, fat to lean ratio and/or estrous cyclicity for use in a subsequent NPB trial of dietary effects on gilt development and retention of sows in the breeding herd to fourth parity. A second objective will be to retain DNA samples from these gilts for genomic analyses of age at puberty.

Materials & Methods:

This experiment took place at the Murphy Brown Sow Research Farm in Milford Utah and used Murphy Brown F1 Maternal Line gilts from their production herd. Twenty four gilts were allocated to each of three pens at 80 days of age on a weekly basis over a 9 week period. Gilts were allocated to pens randomly, except that gilts originating from the same litter or from the same parities were distributed evenly among pens. Gilts were weighed, measured for back fat and loin eye depth using ultrasound, and then started on grower diets containing similar ME levels but differing in SID Lysine levels. Gilts were fed ad libitum, remained on grower diets for 6 weeks, and then were subsequently fed finisher diets differing in SID lysine. Volume of each diet consumed for each pen of gilts was recorded. Table 1 indicates diet formulations for the grower and finisher diets in this experiment. In addition to lowering the SID content, medium and low lysine diets also contained increasing concentrations of corn germ meal to discourage gilts from increasing lysine intake by increasing feed intake.

Gilts on trial were weighed and measured for back fat and loin depth at 28 day intervals from the start of the diets. Beginning at 160 days of age, gilts were exposed to mature boars daily and observed for estrous behavior. Behaviors were recorded according to a 4 point scale, 0 = no outward signs, 1 = red vulva and/or interested in the boar, 2 = red swollen vulva, unopposed boar head to gilt flank, 3 = standing estrus. Day of first standing estrus was considered age at puberty for each gilt. Gilts were observed daily for estrous behavior until 220 days of age. At observed natural estrus, gilts were weighed, measured for back fat and loin eye depth, and the flank to flank distance was measured with a tape measure. At 210 and 220 days of age, a blood sample was collected from all gilts that had not attained puberty (defined as a standing estrus). Blood samples were allowed to clot, centrifuged, and serum was collected. Serum was measured for progesterone by radioimmunoassay to determine whether anestrus gilts were prepubertal or behaviorally anestrus. At 220 days of age, all anestrus gilts were injected with PG600. They were subsequently observed for estrous behavior for an additional week to determine whether they would respond to the injection by attaining puberty. Gilts observed in estrus were recorded and all gilts were then released from the experiment.

Concentrations of progesterone were estimated with a solid-phase RIA (ImmuChem Coated Tube RIA kit, MP Biomedicals, Santa Ana, CA) according to the manufacturers 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. Under these conditions, porcine serum with progesterone measuring 26.1 ng/mL was serially diluted up to 32 fold; concentrations were parallel to the standard curve. When pools of serum from ovariectomized and prepubertal gilts were included, concentrations of progesterone were below the sensitivity of the assay (0.15 ng/mL). A pool of porcine serum 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.

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 body composition, feed intake and feed efficiency included lysine concentration, data recording day, and their interactions and week (1-9) as fixed effects. Body weight at the beginning of the study (i.e. at 100 d of age) was used as a linear covariate in the models to account for the fact that not all gilts started the trial at the same weight. Two analyses were performed for age at puberty. The first analysis included all the gilts that were on trial at 160 d of age. For the second analysis, gilts that were removed from the experiment before 220 d of age were excluded as some of them could have been removed before they reached the physiological maturity required to show standing estrus. Pen within lysine × week was included as a random effect for all the traits analyzed. A backward 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, levels from each main effect were separated using the PDIFF option and a Tukey–Kramer adjustment was used to account for multiple comparisons between levels. Results for fixed effects are reported as least-square means ± SE. Results for continuous variables are reported as the regression coefficient (REG) ± SE.

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. Results are reported as the regression coefficient (REG) ± SE.

Results:

Dietary formulations for this trial are indicated in Tables 1 and 2 for grower and finisher diets, respectively.

On September 6th, 2014, when the first group of gilts was to start boar exposure at 160 days of age, the entire cohort of gilts for this experiment experienced an outbreak of porcine epidemic diarrhea virus. Because of this outbreak, initiation of boar exposure in the first group was delayed 1 week and began with boar exposure for gilts in the second week. No other modifications were made to the protocol in response to this outbreak.

Least squares means for body weight, loin depth, back fat and fat to lean ratio (backfat/loin depth) are presented in table 3, and illustrated in figure 1. Pigs on the high lysine diet reached a mean weight of 148 kg by 212 days of age. Weights were progressively less in medium and low lysine groups compared to the high lysine group as early as 128 days of age, and remained divergent through the remainder of the experiment. Loin depth displayed similar relationships among dietary treatments, and was progressively less in medium and low lysine diets compared to the high lysine diet. In contrast, backfat differed between low and high diets, with the medium diet being intermediate between the two. Despite this, the fat to lean ratio, measured as backfat/loin depth, was not different among dietary groups on any of the days of the trial.

Average daily feed, lysine and metabolizable energy intakes are summarized in Table 4 and illustrated in Figure 2. Daily feed intakes were greater in the low lysine group compared to the high lysine group, with the

medium lysine group being intermediate between the two. Gilts fed the low lysine diet had a lower average daily lysine intake when compared to gilts fed the high lysine diet but did not differ from gilts fed the medium lysine diet. Additionally, average daily lysine intake was similar between gilts fed the medium and high lysine diets. Despite the differences in daily feed intake, daily metabolizable energy intake did not differ among dietary groups throughout the trial.

Average daily gain, feed intake per kg body weight gain, lysine intake per kg body weight gain and metabolizable energy per kg body weight gain are presented in table 5 and illustrated in figure 3. Average daily gain progressively increased with advancing age in all three diets. However, gilts fed the low lysine diet had a lower ADG when compared to gilts fed the medium and high lysine diets Additionally, gilts fed the low lysine diet had a greater feed intake per kg of BW gain compared with gilts fed the high lysine diet but there were no observed differences in the grams of lysine require per kg of BW gain among dietary treatments. Metabolizable energy intake per kg body weight gain generally followed the differences in feed intake per kg body weight gain.

Table 6 indicates the number and percent of gilts that experienced puberty before 220 days of age in each dietary group. Figure 4 illustrates histograms indicating the number of gilts reaching puberty at different ages overall and for each dietary lysine level. Although the number and percent of gilts experiencing puberty progressively increased in the low, medium and high lysine groups, these differences were not statistically significant. However, the percent of gilts reaching puberty overall by 220 days of age averaged 32%, which is atypically poor. Histograms of the distribution of gilts reaching puberty at different ages suggest that the number of gilts reaching puberty may still have been increasing at 220 days of age when the experiment ended, consistent with the low percentage of gilts reaching puberty. It seems likely that the relatively poor incidence of puberty by 220 days of age was due to the PED outbreak. Logistic analysis of the effect of the age of PEDV outbreak on whether a gilt achieved puberty during the trial tended toward significance ($P = 0.09$). However, differences in the incidence of puberty in weekly groups from week 1 to week 9 (Table 7) did not provide a convincing trend for the effect of age at PEDV incidence on puberty incidence.

Table 8 indicates age at puberty for those gilts that experienced puberty before 220 days of age. Average ages at puberty in the high and medium lysine groups did not differ, but were less ($P<0.05$) than for the low lysine group.

Table 9 indicates the number of gilts that were injected with PG600 in each dietary treatment, and the number and percent of gilts that displayed estrus after PG600 treatment. Results indicate that most gilts that had not had a previous standing estrus responded to PG600 with a standing estrus. Table 10 indicates the number of gilts determined to be behaviorally anestrus by progesterone assay of blood samples collected at 210 and 220 days of age. Results indicate that the incidence of behavioral anestrus was low and did not differ among dietary treatments. About 3% of PG600 responder gilts were behaviorally anestrus, in that progesterone concentrations in serum were greater than 1 ng/ml indicating that they had ovulated previous to PG600 treatment. In comparison, 20% of nonresponders had high progesterone in one or more serum samples. Surprisingly, nearly half of the gilts found to be behaviorally anestrus responded to PG600 by displaying estrus.

Tables 11 and 12 summarize gilt removals during the trial. Overall removals, and removals for death, euthanasia, poor body condition, lameness or other reasons did not differ between lysine treatments.

Figure 5 indicates the relationship between body weights and flank to flank measures taken at puberty for each gilt that reached puberty in this experiment. The two measures were correlated ($r=0.77$) as expected, and gilts averaged 115kg at approximately 30 inches flank to flank. Given the results, a threshold of 33 inches flank to flank measure would provide that all gilts were at least 115 kg, however at this threshold the average weight was 150 kg and 20% of gilts exceeded 160 kg.

Table 1. Ingredient list and metabolizable energy and SID lysine level for grower diets (day 100 to day 142) used in this experiment.

Ingredient	High Lysine	Medium Lysine	Low Lysine
Corn, 8.5 CP 425 µ, %	74	58.5	47.8
Soy Meal 47.5 2.9 L, %	16.2	14.4	14.6
Corn Germ NRC 2012, %		8	16
Wheat Midds 18 Starch, %	5	15	18
Dical 21:18.5 P, %	1.56	1.27	1.15
Choice white grease, %	1	1	1
Limestone ground, %	.75	.81	.88
Liq L-Lysine 50%, %	.6	.32	
NaCl, %	.4	.4	.4
Diet Space, %	.2	.2	.2
L-Threonine, %	.14	.04	
Allmet MHA Liq (methionine), %	.1		
Vitamin premix, %	.05	.05	.05
L-tryptophan, %	.03		
Calculated values			
Metabolizable energy kcal/kg	3276	3188	3135
SID Lysine, %	.9	.79	.68

Table 2. Ingredient list and metabolizable energy and SID lysine level for finisher diets (day 143 to 220) used in this experiment.

Ingredient	High Lysine	Medium Lysine	Low Lysine
Corn, 8.5 CP 425 µ, %	80.3	66.1	49.1
Soy Meal 47.5 2.9 L, %	10.3	7.5	7.5
Corn Germ NRC 2012, %		7.5	20
Wheat Midds 18 Starch, %	5	15	18
Dical 21:18.5 P, %	1.38	1.1	.98
Choice white grease, %	1	1	2.9
Limestone ground, %	.77	.9	.93
Liq L-Lysine 50%, %	.45	.29	
NaCl, %	.4	.4	.4
Diet Space, %	.2	.2	.2
L-Threonine, %	.09	.02	
Allmet MHA Liq (methionine), %	.1		
Vitamin premix, %	.03	.05	.05
L-tryptophan, %	.02		
Calculated values			

Metabolizable energy kcal/kg	3278	3192	3208
SID Lysine, %	.68	.60	.52

Table 3. Differences (LS means ± SEM) between lysine levels in the diet by each weight-in date on body composition traits of replacement gilts

	Low		**Medium**		**High**		
	Lsmean	SEM	Lsmean	SEM	Lsmean	SEM	**BW at 100 d**
Body weight (kg)*							
128d	$62.12^{a,1}$	0.69	$65.41^{b,1}$	0.69	$68.67^{c,1}$	0.69	
156d	$79.90^{a,2}$	0.70	$85.78^{b,2}$	0.69	$91.42^{c,2}$	0.69	$1.517(0.055)^{**}$
184d	$103.67^{a,3}$	0.71	$111.16^{b,3}$	0.70	$120.13^{c,3}$	0.70	
212d	$129.83^{a,4}$	0.72	$138.18^{b,4}$	0.72	$148.08^{c,4}$	0.72	
Backfat thickness (mm)*							
128d	$7.03^{a,1}$	0.19	$7.61^{a,b,1}$	0.19	$8.13^{b,1}$	0.19	
156d	$8.87^{a,2}$	0.19	$9.65^{a,2}$	0.19	$10.67^{b,2}$	0.19	$0.149(0.015)^{**}$
184d	$12.43^{a,3}$	0.20	$13.23^{a,3}$	0.19	$14.92^{b,3}$	0.19	
212d	$16.34^{a,4}$	0.20	$16.97^{a,4}$	0.20	$18.82^{b,4}$	0.20	
Loin depth (cm)*							
128d	$3.77^{a,1}$	0.03	$4.10^{b,1}$	0.03	$4.38^{c,1}$	0.03	
156d	$4.22^{a,2}$	0.03	$4.67^{b,2}$	0.03	$5.01^{c,2}$	0.03	$0.045(0.003)^{**}$
184d	$4.86^{a,3}$	0.04	$5.38^{b,3}$	0.03	$5.80^{c,3}$	0.03	
212d	$5.60^{a,4}$	0.04	$6.15^{b,4}$	0.04	$6.55^{c,4}$	0.04	
Fat to lean ratio*							
128d	$1.85^{a,1}$	0.03	$1.84^{a,1}$	0.03	$1.84^{a,1}$	0.03	
156d	$2.09^{a,2}$	0.03	$2.05^{a,2}$	0.03	$2.12^{a,2}$	0.03	$0.009(0.002)^{**}$
184d	$2.54^{a,3}$	0.03	$2.43^{a,3}$	0.03	$2.55^{a,3}$	0.03	
212d	$2.89^{a,4}$	0.03	$2.74^{a,4}$	0.03	$2.86^{a,4}$	0.03	

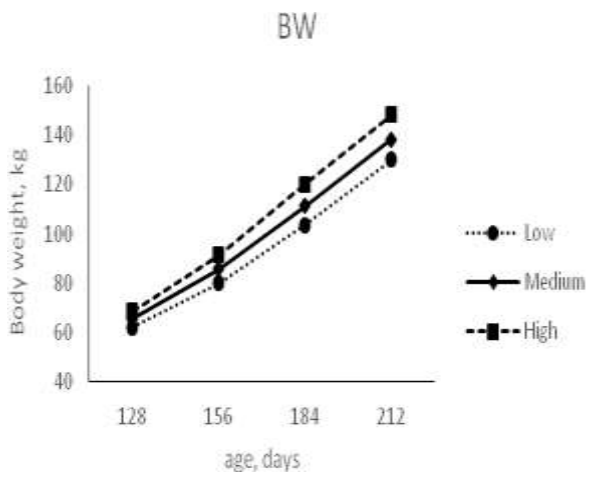
* Significant effect of the interaction between lysine level and weight -in date; $P < 0.05$

** Results for continous variables presented as the regression coefficient and their associate standard error; $P < 0.05$

^{a,b,c} Significant differences between lysine levels; $P < 0.05$

^{1,2,3,4} Significant difference between weight-in dates ; $P < 0.05$

a.



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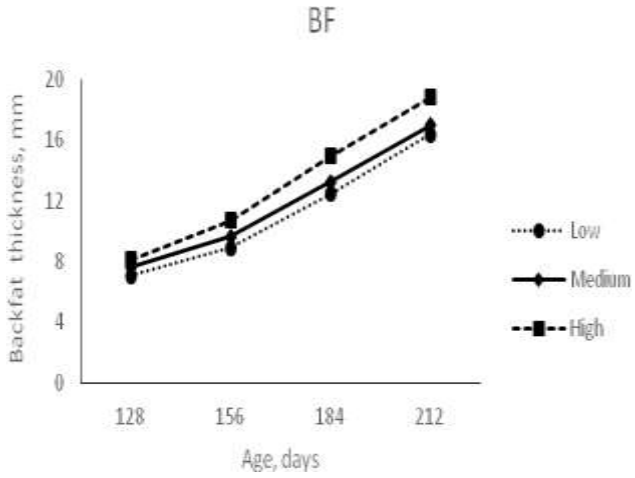

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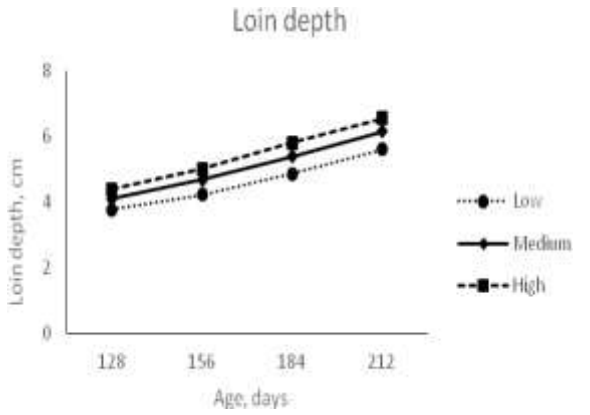

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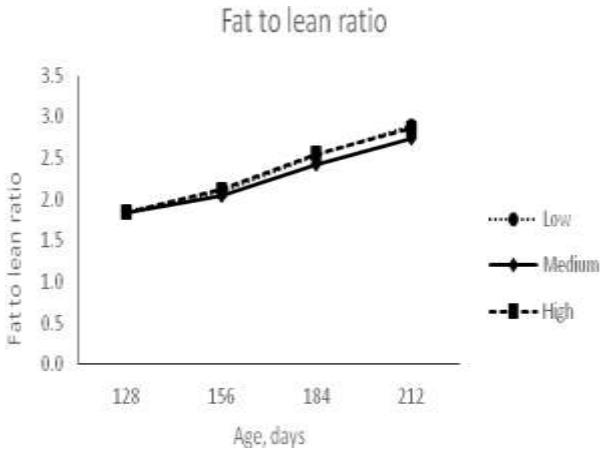


Figure 1. Graphs illustrating least squares means for (a) body weight, (b) back fat, (c) loin depth and (d) fat to lean ratio (back fat divided by loin depth) for gilts fed high, medium and low lysine diets.

Table 4. Differences (LS means ± SEM) between lysine levels in the diet by each weight-in date on feed intake traits of replacement gilts

	Low		**Medium**		**High**		
	Lsmean	SEM	Lsmean	SEM	Lsmean	SEM	**BW at 100 d**
Average daily feed intake (kg)	*ns*[#]						
128d	2.66[a,1]	0.12	2.63[a,1]	0.12	2.56[a,1]	0.11	
156d	2.79[a,1]	0.10	2.64[a,b,1]	0.09	2.46[b,1]	0.09	0.026(0.002)[**]
184d	2.59[a,1]	0.08	2.38[a,b,1]	0.09	2.22[b,1]	0.09	
212d	2.46[a,1]	0.11	2.21[b,1]	0.12	2.18[b,1]	0.14	
Average daily lysine intake (g)	*ns*						
128d	17.87[a,1]	0.82	19.58[a,b,1]	0.79	21.12[b,1]	0.75	
156d	17.09[a,1]	0.64	18.20[a,1]	0.59	19.08[a,1]	0.56	0.169(0.016)[**]
184d	13.37[a,2]	0.55	14.12[a,2]	0.57	15.25[a,2]	0.63	
212d	11.75[a,2]	0.73	12.72[a,b,2]	0.82	15.15[b,2]	0.94	
Average daily ME intake (Mcal)	*ns*						
128d	8.01[a,1]	0.39	8.11[a,1]	0.37	8.17[a,1]	0.35	
156d	8.48[a,1]	0.30	8.21[a,1]	0.28	7.95[a,1]	0.27	0.079(0.007)[**]
184d	7.98[a,1]	0.26	7.45[a,1]	0.27	7.36[a,1]	0.30	
212d	7.54[a,1]	0.34	6.93[a,1]	0.38	7.34[a,1]	0.44	

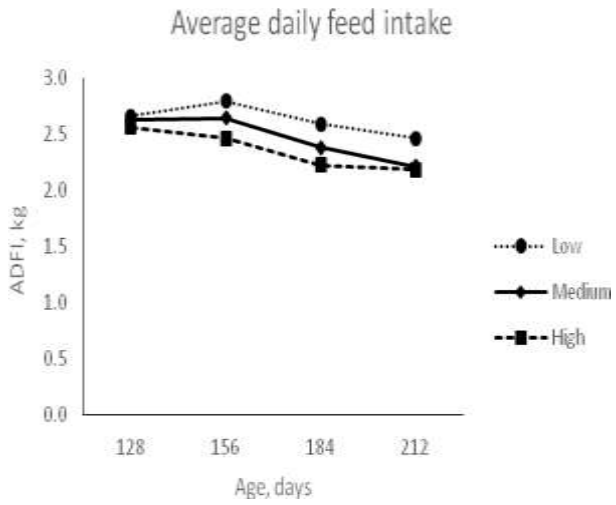
[**] Results for continuous variables presented as the regression coefficient and their associate standard error; $P < 0.05$

[#] ns = non significant effect of the interaction between lysine level and weight-in date ME; $P > 0.05$

[a,b,c] Significant differences between lysine levels; $P < 0.05$

[1,2] Significant difference between weight-in dates ; $P < 0.05$

a.



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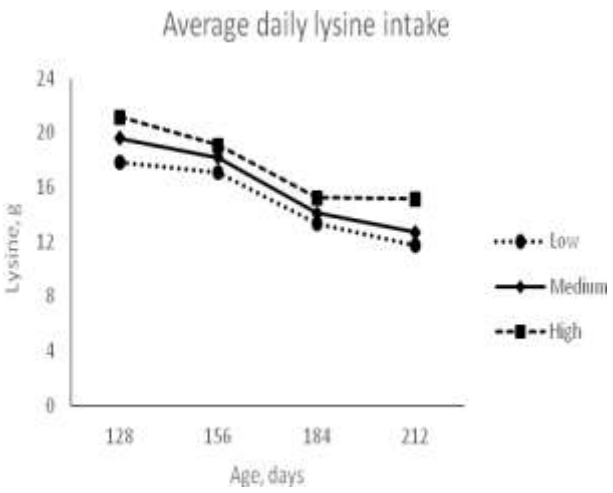

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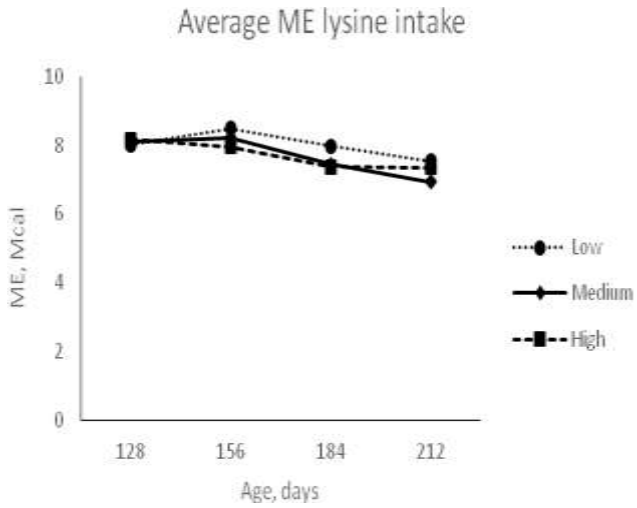


Figure 2. Least squares means for average daily (a) feed, (b) lysine and (c) metabolizable energry intake are illustrated.

Table 5. Differences (LS means ± SEM) between lysine levels in the diet by each weight-in date on feed efficiency traits of replacement gilts

	Low		Medium		High		
	Lsmean	SEM	Lsmean	SEM	Lsmean	SEM	**BW at 100 d**
Average daily gain (kg)	*						
128d	$0.56^{a,1}$	0.02	$0.68^{b,1}$	0.02	$0.79^{c,1}$	0.02	
156d	$0.63^{a,2}$	0.02	$0.72^{b,2}$	0.02	$0.80^{c,2}$	0.02	$0.007(0.001)^{**}$
184d	$0.84^{a,3}$	0.02	$0.90^{a,3}$	0.02	$1.02^{b,3}$	0.02	
212d	$0.92^{a,4}$	0.02	$0.99^{a,4}$	0.02	$0.99^{a,4}$	0.02	
Feed intake per kg of BW gain (kg)	$ns^{\#}$						
128d	$3.13^{a,1}$	0.27	$2.65^{a,b,1}$	0.27	$2.25^{b,1}$	0.27	
156d	$3.89^{a,1}$	0.27	$3.34^{a,b,1}$	0.27	$2.84^{b,1}$	0.27	$NI^{\&}$
184d	$3.22^{a,1}$	0.27	$3.03^{a,1}$	0.27	$2.75^{a,1}$	0.27	
212d	$5.13^{a,2}$	0.27	$4.83^{a,2}$	0.27	$5.08^{a,2}$	0.27	
Lysine intake per kg of BW gain (g)	*ns*						
128d	$21.31^{a,1}$	1.71	$20.96^{a,1}$	1.71	$20.32^{a,1}$	1.71	
156d	$23.43^{a,1}$	1.71	$23.22^{a,1}$	1.71	$22.35^{a,1}$	1.71	NI
184d	$16.77^{a,1}$	1.71	$18.21^{a,1}$	1.71	$18.71^{a,1}$	1.71	
212d	$26.70^{a,2}$	1.71	$28.98^{a,2}$	1.71	$34.55^{b,2}$	1.71	
ME intake per kg of BW gain (Mcal)	ns						
128d	$9.25^{a,1}$	0.85	$8.14^{a,1}$	0.84	$7.26^{a,1}$	0.85	
156d	$11.71^{a,1}$	0.85	$10.37^{a,b,1}$	0.85	$9.21^{b,1}$	0.85	NI
184d	$9.91^{a,1}$	0.85	$9.49^{a,1}$	0.85	$8.99^{a,1}$	0.85	
212d	$15.79^{a,2}$	0.85	$15.11^{a,2}$	0.85	$16.61^{a,2}$	0.85	

* Significant effect of the interaction between lysine level and weight -in date; $P < 0.05$

** Results for continous variables presented as the regression coefficient and their associate standard error; $P < 0.05$

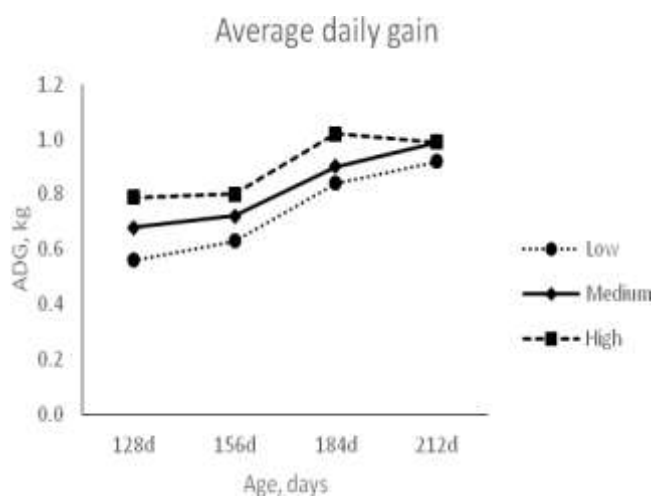
ns = non significant effect of the interaction between lysine level and weight-in date ME; $P > 0.05$

&NI = not included in the model

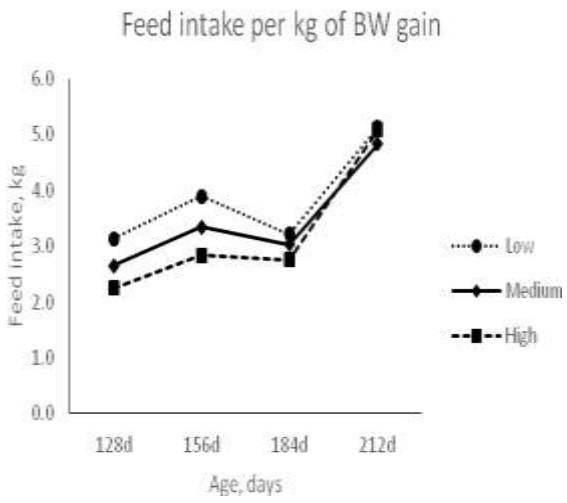
a,b,c Significant differences between lysine levels; $P < 0.05$

1,2,3,4 Significant difference between weight-in dates ; $P < 0.05$

a.



b.



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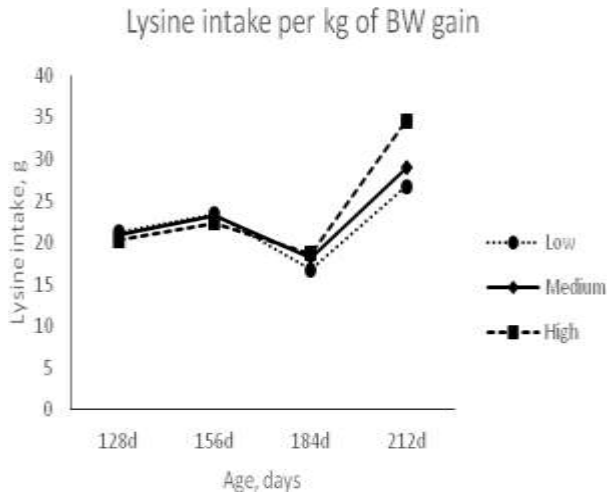

d.

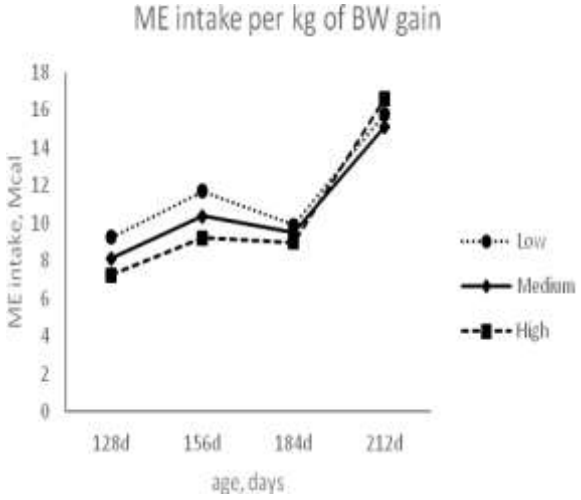

Figure 3. Least squares means for (a) average daily gain, and (b) feed, (c) lysine and (d) metabolizable energy intake per kg of body weight (BW) gain are illustrated.

Table 6. Number and percentage of gilts that showed estrus by each lysine level

	N	Percent
Low	54[a]	27.70
Medium	62	31.00
High	77	37.70

[a] Analysis indicated that number and percentage of gilts did not differ between treatments.

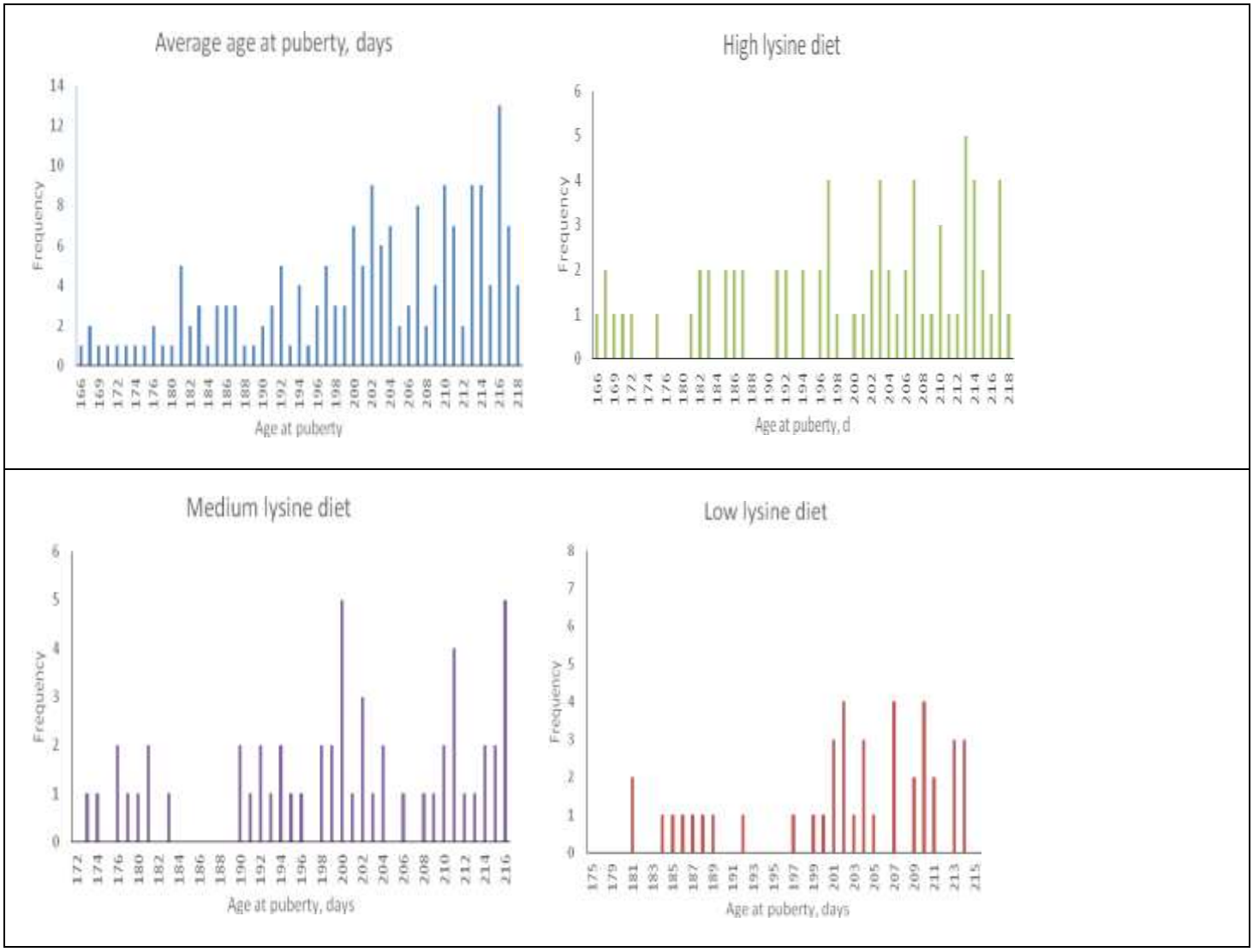

Figure 4: Histograms indicating the number of gilts reaching puberty at different ages for all gilts, and for gilts provided high, medium and low lysine diets are illustrated.

Table 7. Incidence of puberty by 220 days of age in each weekly group of gilts summarized over the three lysine treatments is presented.

Week	Gilts reaching puberty	Number of gilts	Percent reaching puberty
1[a]	19	71	27
2	11	72	15
3	22	71	31
4	19	72	26
5	22	71	31
6	38	70	54
7	28	71	39
8	18	71	25
9	15	72	21

[a]Effect of age at PEDV P=.09.

Table 8. Differences (LS means ± SEM) between lysine levels on age at puberty of replacement gilts.

	Ls mean	**SEM**
Lysine level		
Low	208.99[a]	1.94
Medium	202.29[b]	1.81
High	198.41[b]	1.73

[a,b] Significant differences between lysine levels; $P < 0.05$

Table 9. Number of gilts injected with PG600, the number of gilts that responded by displaying standing estrus within one week, and percentage of the number injected are presented.

	Number injected	**Number responded**	**Percentage per treatment**
High	118	108[a]	91.53
Medium	122	105	86.07
Low	130	112	86.15

[a]Number and percentage responders did not differ between lysine levels

Table 10. Number of gilts that had failed to reach puberty and had progesterone concentrations >1 ng/ml at either 210 or 220 days of age, indicating that they were behaviorally anestrus. Responder and nonresponder refers to displaying estrus in response to injection of PG600 at 220 days of age.

	Responder	Nonresponder	Total
High lysine	3	2	5
Medium lysine	5	3	8
Low lysine	1	5	6
Overall	9	10	19

Table 11. Number and percentage of gilts that were removed (including deaths) by each lysine level

	N	**Percent**
Low	28[a]	36.84
Medium	26	34.21
High	22	28.95

[a]Number and percentage did not differ between treatments.

Table 12. Number of gilts that were removed (including deaths) by each removal reason by each lysine level

	Low	**Medium**	**High**
Dead	15	9	10
Euthanized	0	1	3
Poor body condition	9	11	3
Lameness	2	1	4
Other	2	4	2

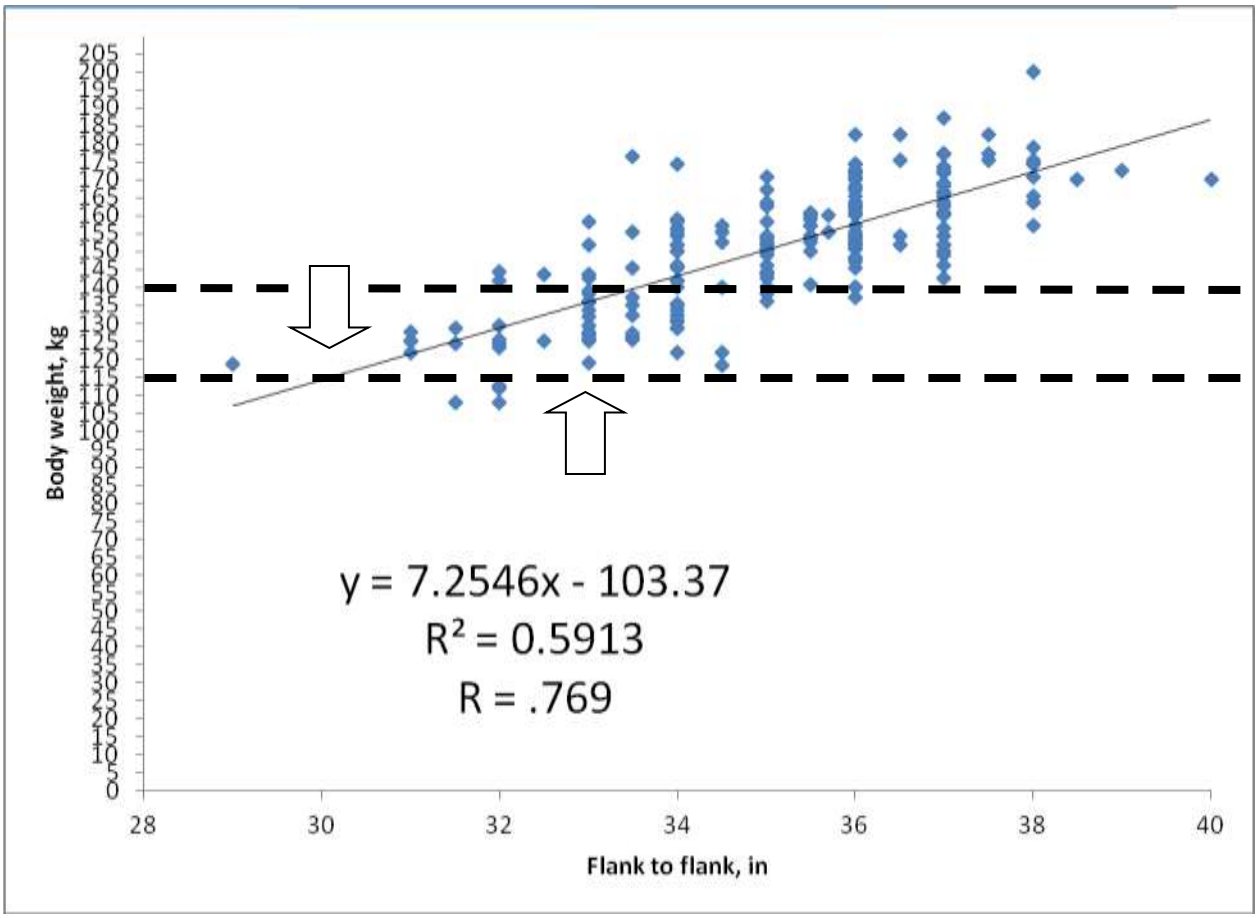

Figure 5. Scatterplot showing the relationship between flank to flank measure and body weights taken at puberty are illustrated. Regression line is indicated by the solid line. A 115 kg minimum weight threshold (lower dashed line) that assumes gilts will grow at 1 kg per day for an additional 20 days for a subsequent estrous cycle is indicated in the small-dashed line, to result in a breeding weight of 135 kg. A 140 kg upper weight threshold (upper dashed line) 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, where all the gilts will be above 135 kg at breeding.

Discussion:

These results indicate that we were successful in developing ad libitum fed gilt diets that alter growth rate in gilts. Despite altering growth rate, the percentage of gilts reaching puberty by 220 days of age was not affected, although the average age at puberty was greater in gilts fed the low lysine diet. One caveat for evaluation of these results is that the gilts became infected with PEDV during the trial, which almost certainly affected the results. This is the most likely explanation for the abnormally low percentage of gilts reaching puberty.

Growth data clearly indicate divergence of body weight growth under the diets applied. Thus, despite having similar metabolizable energy concentrations, progressive reduction in lysine concentrations in the ad libitum fed diets reduced growth. This is in contrast to our previous trial (Calderon Diaz et al., 2015), in which differing lysine concentrations in gilt development diets did not alter growth. The high concentration of SID lysine in the current trial (.9% grower, .68% finisher) was similar to the low concentration in the previous trial (.86% grower, .73% finisher). Results indicate that the lower SID lysine levels used in the current trial compared to the previous trial reduced dietary lysine levels into a range that reduced growth. These results are somewhat lower than previous reports of lysine:metabolizable energy requirements for growing pigs (Main et al., 2008). Main et al., (2008) indicated that a lysine to metabolizable energy ratio of about 3.1 is optimal for gilts that weigh 50 to 70 kg,

which would be when grower diets were fed in this experiment. This compares with a ratio of 2.75 for the high lysine grower diet used here, and 2.4 for the low lysine, high ME grower diet in the previous gilt trial, which did not impair growth of gilts. Daily feed intake reported by Main et al., (2008) was approximately 2 kg, compared to 2.6 kg (at 128 days of age) in this trial and approximately 3 kg per day (at 128 days of age) in the previous gilt trial. Because of this, it is likely that the ratio of lysine:ME in the diet should be adjusted according to the expected intake of the pigs, since the requirement for optimal growth is likely to be a threshold amount of SID lysine consumed per day, rather than a ratio in the diet with metabolizable energy.

In the previous trial, gilts grew to average 168 kg by 212 days of age regardless of diet, compared to 148 kg for the high lysine diet in the current trial at that same age. Gilts also ate less (2.4 kg/day current trial) than in the previous trial (3.2 kg.day). One possible factor explaining this difference in growth between the two sites is that the gilts in the current trial experienced a porcine epidemic diarrhea virus outbreak. This almost certainly impaired both feed intake and growth during the acute phase of the outbreak. This is consistent with previous reports indicating that average daily gain and feed conversion ratio are reduced in pigs after a PEDV outbreak (Alvarez et al., 2015). Within the 9 weekly groups of this experiment, the PED outbreak occurred between 100 (week 9 group) and 160 (week 1 group) days of age. Curiously, average daily gain in the high lysine diet over the entire current trial (0.9 kg/day) was roughly similar to that for the previous experiment (0.9 kg/day), but the trajectory of daily gain was different. In the previous experiment, average daily gain was approximately 1 kg per day until 190 days of age, after which daily gain fell. In the current experiment, average daily gain began low (0.8 through 156 days of age) and then increased to around 1 kg/day. It seems likely that this is indicative of an initial effect of PEDV and then of compensatory gain after the PEDV insult, but the weight reached by 212 days of age suggests that the later gains did not fully compensate for reductions occurring earlier. One other possible factor contributing to differences between the two trials was a difference in the location of the two studies, so it is difficult to assign cause to a single factor. The first trial took place on a research farm in Iowa, the current trial took place at a research farm in Utah. It is possible that some aspect of the Utah site, in addition to the PEDV outbreak, reduced growth and feed intake in gilts. Possible differences between the two sites include local pig density and differences in altitude. The local density of pigs at the Utah site is higher than at the site in Iowa, and it is possible that subclinical disease among pigs may occur more readily in Utah, which may depress growth rates. Alternatively, the two sites differ in altitude. Utah is nearly a mile above sea level (Milford Utah 4967 ft), Iowa is approximately 1000 feet (Goldfield Iowa 1,129 ft). Because of this the two sites differ in oxygen tension, and although pigs are capable of adapting to oxygen tension (Hopkins et al., 2007), perhaps the adaptation is incomplete (Arieli 2008). Very little is known regarding pigs response to altitude with regard to growth performance.

The reduction in growth rate occurred in response to diets without changes in fat to lean ratio, as measured by back fat depth divided by loin depth. This differs somewhat from previous results indicating that changing the lysine to metabolizable energy in the diet of young gilts alters fat to lean ratio (Freisen et al., 1994), but is in agreement with results in older gilts (Freisen et al., 1995). In the Freisen et al., 1994; 1995 experiments, metabolizable energy was held constant and lysine concentrations were varied, and backfat and loin eye area were measured. For gilts from 34 to 72 kg (Freisen et al., 1994), which would be similar to gilts in the current experiment up to 128 days of age, lysine levels of .64% or less increased the fat to lean ratio. The low lysine concentration of .68% in this experiment might have been close to this threshold concentration, but it did not alter the fat to lean ratio. It is possible that this level was either not low enough or it was not fed early enough to alter the fat to lean ratio. A further experiment with gilts from 72 to 136 kg (Freisen et al., 1995), which would be similar to gilts in later stages of the current experiment, indicated no effect of lysine concentration on fat to lean ratio. Thus, the fact that the grower and finisher diets used in the current trial, fed from day 100 to 220 days of age, did not alter fat to lean ratio, is consistent with previous results.

Changes in growth rates did not result in statistically significant changes in the number of gilts that reached puberty by 220 days of age in this experiment, although the age at puberty was greater in gilts fed the low lysine diet. Growth rates are known to affect age at puberty, but previous results indicate that the effects occur at growth rates below .7 kg per day (Bortolozzo et al., 2009). This is consistent with the current results, because the

low lysine diet increased the age at puberty, and growth rates up to 128 days of age were below the .7 kg/day threshold. Growth rates in the medium and high lysine diets exceeded this threshold throughout the experiment. The lack of effect of growth rates on achievement of puberty is also consistent with a previous report of gilts that are limit fed (Kindt et al., 1999), but a subsequent report (Miller et al., 2011) indicated that decreased growth rates under limit feeding did result in both reduction in the number of gilts reaching puberty and increased age at puberty. The lack of difference between treatments in the rate of gilts achieving puberty in the current experiment should be viewed with caution because of the low overall rate of achieving puberty. It is possible that the lack of a significant effect of the diets on puberty achievement may be due to insufficient number of observations given the low overall rate of puberty. Our future planned experiment will have many more observations (~1000 per diet) and will be unlikely to be affected by a PEDV outbreak, since the planned site for our subsequent trial is the Utah research farm, which has already experienced a PEDV outbreak.

Results indicated that most of the gilts that failed to be detected in estrus before 220 days of age were in fact still prepubertal, rather than behaviorally anestrus. Overall, the incidence of behavioral anestrus (inferred by serum progesterone) was low (~5%) and did not differ with lysine concentration in the diet. This conclusion is further supported by the fact that most of the noncycling gilts responded to PG600 treatment by attaining puberty. Curiously, nearly half of gilts found to be behaviorally anestrus by progesterone analysis still responded to PG600 by displaying estrus. These gilts were likely to have been at the stage of their cycle that did not preclude them from responding to PG600, and the PG600 stimulation likely facilitated the display of estrous behavior during the subsequent period of follicular growth and ovulation. This suggests that PG600 treatment of behaviorally anestrous gilts may provide some benefit in terms of stimulating them to display estrus, although it is not clear whether these gilts would be likely to continue to display estrus at the next cycle so that they could be successfully mated.

Weights and flank to flank measures taken at estrus in this experiment provide an opportunity to examine the relationship between flank to flank measures and also assess weights at first estrus with regard to recommended weights at breeding, which would take place at the subsequent estrus event. Recommendations in the literature indicate that gilts should be bred above a threshold of 135 kg (300 lbs; Bortolozzo et al., 2009). Given growth rates of approximately 1 kg per day and assuming a 20 day cycle before breeding, the minimum weight threshold at puberty should be 115 kg, which would correspond to 30 inches for an average weight, or 33 inches if all gilts must meet the minimum weight criterion. If 33 inches is used, 80% of gilts that did not meet the flank to flank threshold were actually above the minimum threshold weight. In fact, despite the reduced growth rates caused by the medium and low lysine diets, all but four gilts met the 115 kg weight criterion at puberty in this experiment. Only one gilt was below 30 inches flank to flank, and her weight actually exceeded the 115 kg threshold. This suggests that the flank to flank measure, because of the correlation of .78, creates errors in both directions, rejecting gilts that are suitable and accepting gilts that are not, depending on the threshold used. Interestingly, even on the low diet in this experiment, and given the average age at puberty that occurred, gilts were likely to reach 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 truly detrimental, this may provide some subsequent advantage during lactation or after weaning for gilts that are developed on the medium or low lysine diets. The average weight at the first estrus in gilts that reached puberty spontaneously in the low lysine diet was 144 kg, close to the upper threshold. For gilts that were induced the average weight at induced puberty was similar (143 kg). This compares to 157 and 159 kg for the high lysine diet. Thus, the low lysine diet could be useful in limiting the number of gilts that exceed a weight threshold at first estrus or breeding, should it be warranted. Our subsequent larger trial to examine litter traits to three parities using these same diets will allow us to confirm whether an upper weight threshold for breeding is warranted, and whether a low lysine diet provides an advantage by reducing the incidence of excessive weight at breeding.

In summary, results indicate that dietary lysine can reduce the growth of ad libitum fed gilts without significantly influencing the number of gilts that reach puberty, but with minor increases in age at puberty. Growth

rate was reduced without altering body composition. The low lysine diet provided for sufficient growth such that most gilts exceeded minimum weight thresholds for breeding, with the possibility of limiting the number of gilts that would have been bred at excessive weights. These diets will be useful for further tests of the consequences of the diets, growth rates and weight thresholds on sow productivity in future experiments that will examine litter production traits to third parity in gilts raised using these same diets.

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