

Effects of increasing phytase in nursery pig diets and
determining the impact of increasing lysine in lactating sows

by

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Abstract

Two experiments using a total of 646 nursery pigs were used to determine the effects of increasing phytase on nursery pig growth performance and bone ash characteristics. Two experiments using a total of 821 sows were used to determine the impact of increasing standardized ileal digestible (SID) lysine (Lys) in lactating sows. Experiment 1 determined the available phosphorus (aP) release of Natuphos E 5,000 G phytase in nursery pigs. Increasing phytase from 0 to 1,000 FTU/kg in phosphorus deficient diets improved nursery pig performance and bone ash characteristics. Using percentage bone ash and formulated phytase concentrations, an equation was developed to predict aP release up to 1,000 FTU/kg of Natuphos E phytase. Experiment 2 was conducted to determine the effect of Superdosing Natuphos E 5,000 G phytase on nursery pig performance and bone ash characteristics. Increasing phytase in diets marginal in P improved pig performance and bone ash values. Increasing phytase in P sufficient diets improved bone ash percent and tended to improve feed efficiency. Experiments 3 and 4 determined the impacts of increasing SID Lys in primiparous and multiparous lactating sows and their litters. In Exp. 3, increasing SID Lys above 0.80% in primiparous sows decreased backfat loss, but had no effect on sow BW loss, ADFI or litter gain. Conception rate at d 30 and percentage born alive tended to improve at 0.95% SID Lys. In Exp. 4 with mixed parity sows, increasing SID Lys to 1.05% increased sow weaning BW, litter gain, and reduced weight loss in lactation. Sow backfat loss increased as SID Lys increased from 0.75 to 1.20%, however loin eye depth loss was reduced as SID Lys increased. Percentage of females bred by d 7 after weaning was improved in primiparous females with increasing SID Lys, however no difference was observed in multiparous sows.

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Dedication

This thesis is dedicated to my grandparents, Richard and Joyce Gourley, and in loving memory of Frank and Helen Klopping.

Chapter 1 - Determining the available phosphorus release of Natuphos E 5,000 G for nursery pigs

Abstract

A total of 286 pigs (PIC 327 × 1050; initially 11.1 ± 0.1 kg, and d 40 of age) were used in a 21-d growth trial to determine the available P (aP) release curve for a novel source of 6-phytase (Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ). Natuphos E is a bacterial derived 6-phytase of which the phytase gene is assembled from a hybrid of phytase-producing bacteria and produced through the fermentation of *A. niger*. Pigs were randomly allotted to pens at weaning. From d 15 to 18 post-weaning, a common corn-soybean meal diet containing 0.12% aP was fed to all pigs to acclimate them to a P-deficient diet. On d 0 of the experiment (d 19 after weaning), pens were allotted in a randomized complete block design to 1 of 8 treatments. There were 4 pigs per pen and 9 pens per dietary treatment. Pigs were fed a corn-soybean meal-based diet formulated to 1.25% standardized ileal digestible Lys. Experimental diets were formulated to contain 0.73% Ca and increasing aP supplied by either monocalcium P (0.12, 0.18 and 0.24% aP) or from increasing phytase (150, 250, 500, 750 and 1,000 FTU/kg) added to the 0.12% aP diet. Analyzed phytase concentrations were 263, 397, 618, 1,100 and 1,350 FTU/kg, respectively. On d 21 of the study, 1 pig per pen was euthanized and the right fibula was collected for bone ash and percentage bone ash calculations. From d 0 to 21, increasing P from monocalcium P or phytase improved (linear, $P < 0.01$) ADG and G:F. Bone ash weight and percentage bone ash increased (linear, $P < 0.01$) with increasing monocalcium P or phytase. When formulated phytase values and percentage bone ash are used as the response variables, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 G phytase can be predicted by the equation: aP release = $0.000212 \times \text{FTU/kg phytase}$.

Key words: bone ash, growth, nursery pig, phosphorous, phytase

Introduction

Phosphorus is an important macro mineral in swine nutrition. Along with Ca and vitamin D, it contributes to bone development and is a component of other physiological functions. Most swine diets are formulated with cereal grains and oilseed meals, which contain 60 to 82% of total P in the form of phytate (Ravindran et al., 1994). Monogastrics do not produce enough enzyme endogenously to cleave the phosphates from the phytate for absorption and consequently much of the phytate-bound P is unavailable to the pig. The ability for a phytase enzyme to improve the available P in swine diets has been well documented (Cromwell et al., 1993; Augspurger et al. 2003; Selle and Ravindran, 2008). As a result, a phytase enzyme is commonly added to diets to make P more available for swine and other animals. This allows for a reduced dietary inclusion of P from inorganic P sources in swine diets and results in reduced P excretion (Simons et al., 1990; Jongbloed et al., 1997).

There are many manufacturers of phytase, and the site in which phosphorus is cleaved from phytate and origin can vary between phytase sources. Although many existing phytase products have already undergone evaluation to determine their unique release curve (Kerr et al., 2010; Jones et al., 2010), new generation phytases are being developed and have not been thoroughly tested to determine their efficacy.

Therefore, the objective for this trial was to evaluate the effects of a novel 6-phytase (Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ) on nursery pig growth performance and bone ash to develop an available phosphorous (aP) release curve.

Materials and Methods

The Kansas State Institutional Animal Care and Use Committee approved the protocol for this study. Ingredients containing Ca or P were analyzed in duplicate prior to manufacturing the diets in order to determine nutrient loading values used for formulation (Table 1-1). Dietary treatments were corn-soybean meal-based and were formulated to meet or exceed NRC (2012) nutrient requirement estimates with the exception of P and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. All diets were formulated to contain the same amount of Ca regardless of increasing aP. Available P coefficients were derived from the 10th edition NRC (1998).

Diet manufacturing started with the production of 10 identical 907 kg batches of basal diet that were packaged in 22.3 kg bags and stored to maintain batch identity (Table 1-2). For each experimental diet, a subset of bags from each basal diet batch was added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 1-3). During bagging of experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, 30th, and 35th bags, and these samples were pooled and used for phytase and nutrient analysis.

The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The nursery barn was environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

A total of 286 nursery pigs (PIC 327 × 1050; initially 11.1 ± 0.1 kg and d 40 of age) were used in a 21-d growth trial. Pigs were initially weaned and randomly allotted to pens and fed common starter diets. On d 15 post-weaning, pens of pigs were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 4 pigs per pen (2 barrows and 2 gilts) and 9 replications (pens) per treatment. From d 15 to 18 post-weaning, a common corn-soybean meal diet

containing 0.12% aP was fed to all pigs to acclimate them to a P-deficient diet. Starting on d 19 post-weaning and continuing for 21 d, pens were fed their respective treatment diets which consisted of 3 diets containing increasing (0.12, 0.18, or 0.24%) levels of aP from inorganic P, provided by monocalcium P, or the 0.12% aP inorganic P diet with 1 of 5 concentrations of added phytase (150, 250, 500, 750, or 1,000 FTU/kg; Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ). The analyzed phytase activity (5,320,000 FTU/kg) was used for determining the amount of phytase to include in each diet.

During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. On d 21 of the study, the median weight gilt in each pen was euthanized via captive bolt. The right fibula was removed from euthanized pigs to determine percentage bone ash criteria. Once collected, all fibulas were stored at -20°C. For processing of fibulas for bone ash, cartilage caps were removed, and bones were boiled for 60 min. Adhering tissue was removed and bones were dried at 105°C for 7 d. Then dried fibulas were ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and calculate percentage bone ash (Flohr et al., 2016).

Chemical analysis

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP (AOAC 990.03, 2006), Ca (AOAC 965.14/985.01, 2006), and P (AOAC 965.17/985.01, 2006) analysis. In addition, ingredients containing Ca and P were analyzed (Ward Laboratories, Kearney, NE) in duplicate prior to manufacturing diets to determine nutrient loading values (Table 1-1). One sample was sent to another commercial laboratory (Eurofins Scientific Inc., Des Moines, IA) and analyzed in duplicate for complete dietary phytase (AOAC 2000.12, 2006).

Statistical analysis

Studentized residuals were evaluated for pen means or individual bone ash measurements to ensure data met the assumption of normal distribution. One pig had a bone ash weight and percentage bone ash 7 SD from the mean and was removed from bone ash analysis, but the pen data were retained for the evaluation of growth data.

Data were analyzed as a randomized complete block design with pen as the experimental unit. An initial base model was evaluated using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Treatment was considered the fixed effect and linear and quadratic contrasts were evaluated within increasing inorganic P or phytase concentrations. Contrast coefficients for phytase concentrations were adjusted to account for the unequal treatment spacing on phytase inclusion.

For pens fed inorganic phosphorus diets, the marginal intake of aP per day was calculated for each pen. The calculation was: dietary aP% minus 0.12% (the aP in the basal diet) multiplied by ADFI. Subsequently, a standard curve was developed for each response criteria using marginal aP release as the predictor variable. The equation for the standard curve was then used to calculate aP release for each pen fed the different phytase treatments based on the observed value for each response criteria. This value was then converted to a marginal aP% using the pen ADFI. Available P release curves were developed for bone ash weight and percentage bone ash.

Mixed model ANOVA with weight block as a random effect was then performed to evaluate aP release as a function of the phytase concentration using linear and quadratic contrasts. Next, mixed model regression was performed to predict aP release as a function of phytase concentration assuming no aP release for the diet containing 0.12% aP and no phytase.

Results were considered to be significant with P -values ≤ 0.05 and were considered marginally significant with P -values ≤ 0.10 .

Results

Chemical analysis

Analyzed CP and P of the experimental diets were similar to those expected from diet formulation. There was some variation in Ca analysis, which increased the Ca:P ratios; however this was unexpected due to the analysis of all major Ca containing ingredients prior to diet formulation. The level of phytase analyzed slightly greater than expected across all diets (Table 1-3). This was unexpected due to the use of the analyzed phytase level for dietary formulation and careful sequencing of diets. Nevertheless, the phytase levels increased in a stepwise fashion with increasing phytase.

Growth performance

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P < 0.001$, Table 1-4) ADG, ending BW, ADFI, and G:F. In addition, pigs fed increasing phytase had improved (linear, $P < 0.001$) ADG, ending BW, ADFI, and G:F.

For bone composition, bone ash weights were increased for pigs fed either increasing inorganic P (linear, $P = 0.003$) or phytase (linear, $P < 0.001$). As a result, percentage bone ash values increased for pigs fed inorganic P (linear, $P = 0.005$) or phytase (linear, $P < 0.001$).

Percentage aP released from this phytase source varied depending on the response criteria (Table 1-5). As phytase concentrations increased, calculated aP increased linearly ($P < 0.001$) to the highest phytase concentration for all response criteria. However, the rate of increase from the prediction equation varied by response variable with a release of 0.159% aP for bone ash weight and 0.227% aP for percentage bone ash at 1,000 FTU/kg. Based on the linear response for aP

release associated with percentage bone ash, a prediction equation ($\text{aP release} = 0.000212 \times \text{FTU/kg}$) was developed that predicts the aP release at different dietary phytase concentrations.

Discussion

Phosphorous is a key mineral in animal diets for bone development and other physiological functions. However, the majority of P in cereal grains and oilseeds commonly fed to swine is bound in the form of phytate and not available for absorption (Ravindran et al., 1994). Swine are unable to cleave P from phytate because they produce insufficient amounts of endogenous phytase in their small intestine (Jongbloed et al., 1992; Humer et al., 2015). While microflora activity in the large intestine produces larger amounts of endogenous phytase, absorption of P takes place in the small intestine, thus P released in the large intestine will be excreted (Smith et al., 1955; Bohlke et al., 2005, Rutherford et al., 2014).

Commercially produced microbially-derived phytase is one of the most significant enzyme discoveries used in swine diets (Cromwell, 2009). The phytase enzyme (*myo*-inositol hexaphosphate phosphohydrolase) catalyzes the hydrolysis of phytate to inorganic phosphate (PO_4) and *myo*-inositol (Humer et al., 2015). While intermediate products are synthesized in the stepwise dephosphorylation reaction, only *o*-phosphate ions (PO_4) can pass through the gastrointestinal wall and be utilized by the animal (Jongbloed et al., 1992). Phytase inclusion in swine diets allows more dietary P to be absorbed in the proximal end of the small intestine and results in less excretion of P from the pigs (Gonzalez-Vega and Stein, 2014).

Phytase activity is measured in the form of phytase units (FTU). One FTU is defined as the quantity of phytase enzyme required to liberate 1 micromol of inorganic P per minute, at pH 5.5, from an excess of 15 micromol per L of sodium phytate at 37°C (AOAC, 2006). A common method to evaluate the efficacy of a phytase source is to determine the phytase activity needed to

reach a specific aP release value in the diet (Goncalves et al., 2016). Several microbial phytase sources are available for swine producers, yet each source can have a different aP release value (Jones et al., 2010). Consequently, it is important to determine aP release for each specific phytase source and compare to other sources on an equal FTU inclusion basis.

The previous Natuphos product (Natuphos) was a 3-phytase and was derived from fermentation of *A. niger*. The new generation, Natuphos E, is a bacterial derived 6-phytase of which the phytase gene is assembled from a hybrid of phytase-producing bacteria and produced through the fermentation of *A. niger*. Currently, literature is limited regarding the use of Natuphos E in swine diets. Torallardona and Ader (2016) conducted a 42-d study to determine growth performance, bone ash values, and ATTD for P in nursery pigs fed 125 to 1,000 FTU/kg Natuphos E in P-deficient diets. Over the entire 42-d study, increasing Natuphos E improved (linear, $P < 0.03$) ADG, ADFI, G:F and bone characteristics compared to pigs receiving a P-deficient diet with no phytase. These findings are in agreement with the current study, where ADG, ADFI and bone ash values increased linearly in P-deficient diets when phytase inclusion increased from 150 to 1,000 FTU/kg. Torallardona and Ader (2016) further observed that increasing phytase improved (linear and quadratic, $P \leq 0.026$) ATTD for P, Ca and ash with the greatest improvement occurring up to 250 FTU/kg.

Linear improvements in growth performance and bone characteristics were observed when P-deficient diets (0.12% aP) were supplemented with increasing aP provided by monocalcium phosphate. Previous research has shown P-deficient diets reduced feed efficiency and bone ash values in weanling pigs (Mahan, 1982). Furthermore, Augspurger et al. (2003) demonstrated that feed efficiency and bone ash linearly improved as inorganic P (KH_2PO_4) was added to a basal diet formulated to be low in aP (0.075% aP), which supports our findings of

increased feed efficiency and percentage bone ash with increasing monocalcium phosphate in a P-deficient diet.

Kornegay and Qian (1996) evaluated the addition of phytase to P-deficient diets to determine the aP release value of a phytase product. They determined that ADG, apparent P digestibility, and ash content of bone were the more sensitive indicators to develop aP release values. In the current study, aP release values for performance criteria (ADG and G:F) were lower than the release values for percentage bone ash, which might be a result of the elevated analyzed Ca concentrations. The NRC (2012) cites the total Ca requirement estimate for an 11 to 25 kg growing pig to be 0.70%, and our diets were formulated to contain 0.73% total Ca and analyzed to approximately 0.75 to 0.89% Ca. A recent study by Gonzalez-Vega et al. (2016) demonstrated that as STTD Ca was increased from 0.32 to 0.72% in nursery pig diets, growth criteria (ADG and G:F) worsened (linear, $P < 0.05$). Conversely, percentage bone ash increased (quadratic, $P < 0.05$) as dietary Ca increased in the diet. This is in agreement with the growth performance from the current study, where aP release values were lower for ADG and G:F. However, bone ash weight and percentage bone ash did not seem to be effected by the total Ca values as Gonzalez-Vega et al. (2016) would suggest. As a result, bone ash weight and percentage bone ash were used to predict aP release values.

In the present study, when using percentage bone ash to predict an aP release curve, the aP release per FTU/kg in the diet is less than suggested by the manufacturer. This could be due to differences in type of bone (fibula vs. metatarsals) used for bone analysis in which an aP release curve was developed from. Fibulas are easier to remove intact and are easier to clean consistently compared to metatarsals, which allows for greater bone ash values (Biehl and Baker, 1996). Another consideration is type of feedstuffs included in the diet in which the aP release

curve was developed. Depending on the type of cereal grain used in formulation and the amount of phytate bound P or phytase already in the grain, the phosphorus release could vary. It is suggested that the magnitude of response to phytase is correlated with the level of dietary phytate (Selle and Ravindran, 2008).

In summary, this study has provided an aP release curve that can be used for Natuphos E 5,000 phytase as a source of aP in nursery diets when included at concentrations between 150 and 1,000 FTU/kg. Using percentage bone ash as the response criteria, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 can be predicted by the equation: aP release = $0.000212 \times \text{FTU/kg}$ phytase. Further research needs to be conducted to determine aP release of Natuphos E when included in grower and finisher diets and in diets containing levels of phytase above 1,000 FTU/kg.

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Table 1-1 Analyzed ingredient composition (as-fed basis)¹

Ingredient	Ca, %	P, %
Corn	0.04	0.37
Soybean meal	0.41	0.82
Limestone	36.79	0.01
Monocalcium P	16.85	22.22
Vitamin premix	17.51	0.02
Trace mineral premix	18.43	0.06

¹Two samples of each ingredient were pooled and analysis was performed by two commercial laboratories in duplicate (Ward Laboratories, Kearney, NE and Cumberland Valley Analytical Services, Hagerstown, MD).

Table 1-2 Composition of basal batch (as-fed basis)^{1,2}

Ingredient	%
Corn	63.67
Soybean meal, 48% CP	33.85
Monocalcium P, 22% P	0.20
Limestone	1.04
Sodium chloride	0.35
L-Lys-HCl	0.30
DL-Met	0.12
L-Thr	0.12
Trace mineral premix ³	0.15
Vitamin premix ⁴	0.25
	100

Calculated analysis

Standardized ileal digestibility (SID) amino acids, %

Lys	1.25
Ile:Lys	63
Leu:Lys	129
Met:Lys	33
Met & Cys:Lys	57
Thr:Lys	63
Trp:Lys	18.7
Val:Lys	69
Total Lys, %	1.40
CP, %	21.8
ME, kcal/g	3,353
NE, kcal/g	2,464
SID Lys:ME, g/Mcal	3.78
Ca, %	0.64
P, %	0.54
Available P ⁵ , %	0.12
STTD P, %	0.24

¹The basal batch was used as the major ingredient within each experimental diet.

²Analyzed Ca and P values were used in formulation.

³Provided per kilogram of premix: 26.5 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc sulphate, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

⁴Provided per kg premix: 4,409,171 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 15 mg vitamin B12; 1,764 mg menadione; 3,307 mg riboflavin; 11,023 mg pantothenic acid, 19,841 mg niacin.

⁵Coefficients for formulation were derived from NRC (1998).

Table 1-3 Ingredient composition of experimental diets (as-fed basis)

Ingredient, %	Experimental diet							
	Inorganic P			Phytase ¹				
	0.12%	0.18%	0.24%	150	250	500	750	1,000
Basal batch	99.01	99.01	99.01	99.01	99.01	99.01	99.01	99.01
Limestone	0.25	0.13	---	0.25	0.25	0.25	0.25	0.25
Monocalcium P	---	0.27	0.54	---	---	---	---	---
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sand ²	0.34	0.20	0.05	0.34	0.34	0.33	0.33	0.32
Phytase	---	---	---	0.003	0.005	0.009	0.014	0.019
	100	100	100	100	100	100	100	100
Calculated analysis								
CP, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
P, %	0.54	0.60	0.66	0.54	0.54	0.54	0.54	0.54
Phytase, FTU/kg	---	---	---	150	250	500	750	1,000
Ca:P ratio	1.35	1.22	1.11	1.35	1.35	1.35	1.35	1.35
Analyzed composition ³								
CP, %	21.5	19.8	22.0	21.4	22.2	22.9	22.1	23.1
Ca, %	0.75	0.79	0.87	0.77	0.82	0.89	0.80	0.86
P, %	0.50	0.57	0.64	0.49	0.50	0.48	0.50	0.51
Phytase, FTU/kg	95	< 60	< 60	263	397	618	1,100	1,350
Ca:P ratio	1.50	1.39	1.35	1.57	1.64	1.85	1.60	1.68

¹Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) was analyzed for phytase level, and it contained 5,320,000 phytase units (FTU)/kg.

²Sand was used to equalize inclusion rate of the basal batch with experimental ingredients.

³Seven samples per dietary treatment were pooled and used to create a composite sample. One composite sample was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP, Ca and P analysis. Another composite sample was sent to a commercial laboratory (Eurofins Scientific Inc., Des Moines, IA) and analyzed in duplicate for complete dietary phytase.

Table 1-4 Effects of increasing aP from inorganic P or Natuphos E 5,000 G on nursery pig growth performance and bone ash values¹

Item	Inorganic P, aP % ²			Phytase, FTU/kg ³					SEM	Inorganic P		Phytase	
	0.12	0.18	0.24	150	250	500	750	1,000		Linear	Quadratic	Linear	Quadratic
BW, kg													
d 0	11.1	11.2	11.2	10.9	10.9	11.0	11.1	11.2	0.19	0.724	0.975	0.126	0.133
d 21	20.3	22.2	23.4	21.3	21.6	21.6	22.5	23.3	0.38	<0.001	0.478	<0.001	0.906
d 0 to 21													
ADG, g	434	535	584	488	495	501	541	575	13.7	<0.001	0.111	<0.001	0.666
ADFI, g	858	936	981	916	901	896	966	970	21.0	<0.001	0.517	<0.001	0.959
G:F, g/kg	505	572	596	532	555	561	561	590	10.1	<0.001	0.084	<0.001	0.204
Bone ash weight, g ⁴	0.678	0.850	0.856	0.713	0.666	0.769	0.819	0.936	0.041	0.003	0.103	<0.001	0.194
Bone ash, % ⁴	38.1	41.2	42.1	38.7	39.7	41.4	43.2	45.6	1.01	0.005	0.332	<0.001	0.614

¹A total of 286 nursery pigs (PIC 327 × 1050; initially 11.1 kg and d 40 of age) were used in a 21-d growth study evaluating the effects of increasing available P from inorganic P or from a novel phytase source.

²Inorganic P was added to the diet by increasing monocalcium P.

³Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ).

⁴One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

Table 1-5 Calculated aP release values based on different response criteria

Item	Phytase, FTU/kg ¹					SEM	Probability, <i>P</i> <	
	150	250	500	750	1,000		Linear	Quadratic
ADG	0.036	0.042	0.050	0.079	0.103	0.009	0.001	0.325
G:F	0.025	0.046	0.072	0.064	0.109	0.014	0.001	0.226
Bone ash weight	-0.003	-0.036	0.042	0.073	0.159	0.008	0.001	0.206
Percent bone ash	0.000	0.034	0.093	0.144	0.227	0.032	0.001	0.737

¹Natuphos E 5,000 G FTU/kg (BASF Corporation, Florham Park, NJ).

Table 1-6 Available P release equations for Natuphos E 5,000 phytase based on various response criteria

Response	aP release equation
Bone ash weight	$\text{aP release} = 0.000116 \times \text{FTU/kg}$
Percentage bone ash	$\text{aP release} = 0.000212 \times \text{FTU/kg}$

Chapter 2 - Effects of high doses of Natuphos E 5,000 G phytase on growth performance of nursery pigs

Abstract

A total of 360 pigs (DNA 200 × 400, initially 5.9 ± 0.1 kg) were used in a 42-d trial to determine the effect of high doses of a novel phytase source (Natuphos E 5000 G, BASF Corporation, Florham Park, NJ) on nursery pig growth and bone ash. Pigs were randomly allotted to pens at weaning and pens were allotted to 1 of 8 dietary treatments in a randomized complete block design. There were 5 pigs per pen and 9 pens per treatment. Diets were fed in 3 phases (d 0 to 7, 7 to 21, and 21 to 42) with formulated total Ca:P of 1.07, 1.05, and 0.93, respectively. Treatments included a negative control (NC) with 0.40, 0.30, or 0.25% aP from monocalcium P for Phases 1, 2, and 3 respectively; and NC with either 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg phytase. The last two treatments were a positive control (PC) with 0.55, 0.45, or 0.40% aP from monocalcium P for Phases 1, 2, and 3, respectively, or PC with 2,000 FTU/kg phytase. The NC diet with 500 FTU/kg and PC without added phytase were formulated to be equivalent in available Ca and P. On d 42, one pig per pen was euthanized and the right fibula was removed for bone ash analysis. From d 0 to 42, pigs fed increasing phytase in the NC tended to have increased (quadratic, $P = 0.064$) ADG and (linear, $P = 0.082$) ending BW and had improved (quadratic, $P = 0.008$) G:F. Adding 2,000 FTU/kg phytase to the PC did not influence ADG or ADFI, but tended to improve ($P = 0.060$) G:F compared with the PC. In addition, percentage bone ash increased as phytase increased in the NC (linear, $P < 0.001$) or when 2,000 FTU/kg was added to the PC diets ($P < 0.001$). Pigs fed the PC had increased ($P = 0.007$) ADFI and tended to have greater ($P = 0.099$) percentage bone ash than pigs fed NC+500 FTU/kg phytase, but the pigs fed NC+500 FTU/kg phytase had improved ($P = 0.032$) G:F compared to

pigs fed the PC. In summary, increasing concentrations of dietary phytase in a P deficient diet improved growth and bone ash measurements and was optimized at 1,000 FTU/kg. There were varied improvements when 2,000 FTU/kg was added in P adequate diets.

Key words: bone ash, calcium, growth, nursery pig, phosphorus, phytase

Introduction

Phytase enzymes have been commercially available for use in monogastric diets since the early 1990's (Adeola and Cowieson, 2011). Cereal grains and oilseeds can contain 60 to 82% of total phosphorous in the form of phytate-bound P (Ravindran et al., 1994). Because the pig cannot produce enough endogenous phytase for P absorption, a phytase enzyme is commonly added to cleave the phosphate from the phytate for complete absorption. Phosphorus, along with Ca, is an important macro mineral that contributes to bone development and other physiological functions. The ability for a phytase enzyme to improve the available P in swine diets has been well documented (Cromwell et al., 1993; Augspurger et al., 2003; Selle and Ravindran, 2008). The addition of phytase also allows for reduced inclusion of inorganic P, and consequently reduces P excretion from the pig (Simons et al., 1990; Jongbloed et al., 1997).

Previous studies have shown improved growth performance in nursery pigs fed high concentrations of phytase at or above 10,000 FTU/kg (Kies et al., 2006; Nyannor et al., 2007; Zeng et al., 2014). The suggested mode of action for high concentrations of phytase comes in the form of non-P related benefits from improved digestibility of energy, AA, and other minerals (Kies et al., 2001). However, it is noted that greater growth performance improvement is seen when digestible P, AA, and other nutrients are at marginal concentrations relative to the dietary predicted requirements (Goncalves et al., 2016).

Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) is a relatively new source of phytase available to the U.S. swine industry. In a previous study (Gourley et al., 2016), Natuphos E 5,000 G improved (linear, $P < 0.01$) ADG, ADFI, G:F, and percentage bone ash as phytase increased from 0 to 1,000 FTU/kg. However, current literature is not available to determine the impact of feeding concentrations above 1,000 FTU/kg of this new phytase source. Therefore, the objective of this study was to evaluate the effect of high doses of Natuphos E 5,000 G on the growth performance and percentage bone ash in nursery pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the Kansas State University Segregated Early Wean Facility in Manhattan, KS. Two identical barns were environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

Ingredients containing Ca or P were analyzed in duplicate prior to manufacturing the diets in order to determine nutrient loading values used for formulation (Table 2-1). Dietary treatments were corn-soybean meal-based and were formulated to meet or exceed NRC (2012) nutrient requirements with the exception of P and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The analyzed phytase activity (5,111,000 FTU/kg) was used for determining the amount of Natuphos E 5,000 G to include in each diet.

Dietary treatments included a negative control with 0.40, 0.30, or 0.25% aP (0.44, 0.36, 0.32 % standardized total tract digestible [STTD] P) from inorganic P, provided by monocalcium P, for Phases 1, 2, and 3, respectively. Additional dietary treatments included the negative

control plus increasing phytase at 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ) in each phase; a positive control with 0.55, 0.45, or 0.40% aP (0.57, 0.49, 0.46 % STTD P) from inorganic P for Phases 1, 2, and 3, respectively, or the positive control with 2,000 FTU/kg of phytase in each phase. The positive control was formulated with Ca and P similar to current industry levels, which resulted in Ca being close to NRC (2012) requirement estimate, but P was formulated above the NRC (2012) estimated requirement for the weight range corresponding to each phase. The NC was formulated to be the PC minus 0.15% P and 0.14% Ca, which was the amount the manufacturer suggested would be released by 500 FTU/kg Natuphos E 5,000 G. Available P coefficients were derived from the 10th edition NRC (1998). Using STTD P values, the NC was also below the NRC (2012) requirement estimates, while the PC was formulated well above the STTD P estimates.

All dietary treatments within phase were derived from a basal batch of ingredients (Table 2-2). After manufacturing the basal batch, they were bagged off into 8 identical sets (89 kg of Phase 1, 357 kg of Phase 2, and 893 kg of Phase 3 per treatment). For each experimental diet, a subset of bags from the basal batch was added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 2-3). During bagging of experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, and 35th bags, pooled, and used for phytase and nutrient analysis.

A total of 360 barrows (DNA 200 × 400; initially 5.9 ± 0.1 kg and 21 d of age) were used in a 42-d growth trial. Pigs were randomly allotted to pens and then pens of pigs were blocked by weight and randomly allotted to 1 of 8 dietary treatments. There were 5 pigs per pen and 9 replications (pens) per treatment. Diets were fed in 3 phases from d 0 to 7, 7 to 21, and 21 to 42. During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and

G:F. On d 42 of the study, the median weight pig in each pen was euthanized via captive bolt and fibulas were collected to determine bone ash values. Once collected, all fibulas were stored at -20°C. To determine bone ash concentrations, bones were autoclaved for 60 min. Adhering tissue and cartilage caps were removed and bones were dried at 105°C for 7 d. Then dried fibulas were ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and percentage bone ash.

Chemical analysis

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP (AOAC 990.03, 2006), Ca (AOAC 965.14/985.01, 2006), and P (AOAC 965.17/985.01, 2006) analysis (Table 2-4). In addition, ingredients containing Ca or P were analyzed (Ward Laboratories, Kearney, NE) in duplicate prior to manufacturing diets to determine nutrient loading values. One sample per treatment was sent to another commercial feed laboratory (Eurofins Scientific Inc., Des Moines, IA) for complete diet phytase analysis (AOAC 2000.12, 2006).

Data analysis

All data (pen means or bone values) 3 SD outside the mean of each response criteria were evaluated as outliers. A subsequent investigation showed that the outliers in this study were due to a greater number of pigs removed in a few pens, thus they were removed from analysis. In Phase 1, there were 4 pen outliers for G:F, 1 G:F outlier for Phase 2, and 1 G:F outlier for Phase 3. However, the pen data were retained for the evaluation of bone analysis data.

Data were analyzed as a randomized complete block design with pen as the experimental unit. Barn was treated as a random effect. Contrast coefficients for phytase concentrations were adjusted to account for the unequal treatment spacing on phytase inclusion. Pre-planned contrast

statements were used to determine the linear and quadratic responses to phytase. A pairwise comparison was used to compare the PC and PC + 2,000 FTU phytase treatments to test for an extra phosphoric effect. Another pairwise comparison was used to compare the NC + 500 FTU/kg and the PC control to confirm the estimated release of Natuphos E 5,000 G. A third pairwise comparison was used to compare the NC and the PC. Analysis of variance was performed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Results were significant with P -values ≤ 0.05 and were considered marginally significant with P -values > 0.05 and ≤ 0.10 .

Results

Chemical analysis

Analysis of CP and P of the experimental diets were similar to those expected from diet formulation, however Ca in the final diets analyzed greater than expected. This was not anticipated since all ingredients containing Ca were analyzed and those values were used in diet formulation. Analyzed phytase increased as phytase addition increased as anticipated, but was greater than expected across all diets (Table 2-4).

Growth performance

From d 0 to 7 and 7 to 21, there were no differences observed for growth performance among pigs fed any of the dietary treatments (Table 2-5). From d 21 to 42, increasing phytase tended to increase (quadratic, $P = 0.078$) ADG and (linear, $P = 0.095$) ADFI. In addition, G:F improved (quadratic, $P = 0.001$) with increasing phytase. When comparing the NC diet with 500 FTU/kg phytase and the PC diet formulated to have the same aP, pigs fed the PC diet had increased ($P < 0.05$) ADG and ADFI; however, pigs fed the NC with 500 FTU/kg of phytase had improved ($P = 0.047$) G:F. Among pigs fed the 2 positive control diets, including phytase at

2,000 FTU improved ($P = 0.047$) G:F. Pigs fed the PC had increased ($P = 0.038$) ADG and ($P = 0.049$) ADFI compared to those fed the NC.

From d 0 to 42, pigs fed increasing phytase tended to have increased (quadratic, $P = 0.064$) ADG resulting in heavier (linear, $P = 0.082$) ending BW and improved (quadratic, $P = 0.008$) G:F. Pigs fed the NC diet with 500 FTU/kg phytase and PC diets were formulated to be equivalent in available Ca and P. When comparing these diets, pigs fed the positive control diet had increased (linear, $P = 0.007$) ADFI; however, pigs fed the NC with 500 FTU/kg phytase diet had improved (linear, $P = 0.032$) G:F. Adding 2,000 FTU/kg phytase to the positive control diet did not influence ADG or ADFI, but tended to improve ($P = 0.060$) G:F. Pigs fed the NC had poorer ($P \leq 0.030$) ADG and ADFI compared to the PC diet, but no difference in G:F was observed.

Pigs fed increasing phytase had increased bone ash weights (quadratic, $P < 0.001$). In addition, percentage bone ash values increased (linear, $P < 0.001$) as phytase increased. There was a tendency for pigs fed the PC diet to have greater ($P = 0.099$) percentage bone ash when compared to the NC diet containing 500 FTU/kg of phytase. Pigs fed the PC diet with phytase had increased ($P = 0.001$) percentage bone ash compared to when pigs were fed the PC diet without phytase. Finally, pigs fed the PC diet had greater ($P < 0.010$) bone ash weight and percentage compared to pigs fed the NC diet.

Discussion

Commercially produced microbially-derived phytase is one of the most significant enzyme discoveries used in swine diets (Cromwell, 2009). Since the early 1990's, it has been used to efficiently make P, that is bound in the form of phytate, available to monogastrics. Many commercial phytases are available for use in swine diets; however, phytase enzymes differ based

on the origin, specificity and configuration (Rodehutsord and Rosenfelder, 2016). Thus, each product should have its own unique available P release curve to be used in formulation. Many products have already undergone studies to determine specific phytase release curves (Kornegay and Qian, 1996; Kerr et al., 2010; Jones et al., 2010). Recently, Gourley et al. (2016) determined the available P release curve for Natuphos E 5,000 G. When using concentrations between 150 and 1,000 FTU/kg and utilizing percentage bone ash as the response criteria, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 was predicted by the equation: aP release = $0.000212 \times$ FTU/kg phytase.

Based on the linear response to increasing Natuphos E up to 1,000 FTU/kg (Gourley et al., 2016), the current study aimed to evaluate growth performance and bone ash when adding phytase above 1,000 FTU. The current study revealed a quadratic increase for growth performance (ADG and G:F) up to 1,000 FTU/kg of phytase in the NC, with no further improvement when included up to 4,000 FTU/kg phytase. Few studies are available on the effects of Natuphos E, and to our knowledge this is the first study to demonstrate high concentration release values of Natuphos E. Kornegay and Qian (1996) observed that with an older generation of Natuphos, breakpoints for growth performance were between 750 and 1,050 FTU/kg. The total P levels in the current experiment NC diets were slightly below the NRC (2012) requirement estimate for each nursery phase. In a recent study Vier et al. (2016) formulated diets from 80 to 160% of the NRC (2012) STTD P requirement estimate and determined that growth was linear up to 160% for a 15 to 25 kg pig, which would suggest that pigs were still below the P requirement needed to maximize growth performance. The quadratic response to phytase in the current study could be explained in part by releasing maximum P at

1,000 FTU/kg to optimize growth performance, with no additional benefit in growth performance when more phytate-bound P was released.

The current study showed a linear increase in bone ash weight and percentage bone ash as phytase increased from 0 to 4,000 FTU/kg. Our study would suggest that the requirement to improve percentage bone ash is greater than what is needed to maximize growth performance in the pig. This is like other studies (Kornegay and Thomas, 1981; Mahan, 1982) that observed the P and Ca requirement to maximize bone development is greater than the requirement for growth performance. However, there was no indication that the amount needed for maximum bone development influences structural soundness.

Kies et al. (2006) observed an improvement in growth performance and digestibility of minerals when phytase (Natuphos) was included up to 15,000 FTU/kg in P-deficient diets. Similarly, Zeng et al. (2014) also observed improved growth performance, mineral digestibility and bone ash weight as phytase (Phyzyme XP) increased up to 20,000 FTU/kg in P-deficient diets. Because the P requirement would be met at a low addition of phytase, it is suggested that the additional benefit in performance is not coming from P, but rather a release of AA, energy, and other minerals (Selle and Ravindran, 2008). Beers and Jongbloed (1992) were the first to observe an improvement in growth performance when phytase was included in P-sufficient diets, again suggesting the improvement in growth was due to increased digestibility of other nutrients rather than of P. The current study would disagree with these results, where phytase added at 2,000 FTU/kg in a P-sufficient diet did not provide a benefit in ADG or ADFI; however, there was a tendency observed for an improvement in G:F.

A review by Adeola and Cowieson (2011) suggests that when phytate is present in the gut, AA, vitamins and minerals, energy viability and absorption are reduced. Phytate:protein

complexes can form due to an electrostatic attraction between molecules, which can reduce the amount of AA available for absorption. In addition, intact phytate that reaches the duodenum will seek out divalent cations, such as Ca, and create insoluble precipitates where its absorption is reduced (Cowieson et al., 2009). The reduction of Ca from these precipitates further reduces the ability for endogenous processes to proceed and can negatively impact pig performance. Therefore, it is thought that phytase could help to release nutrients other than P that are unavailable to the pig due to high concentrations of phytate.

Providing high concentrations of phytase is also suggested to influence *myo*-inositol availability for the pig. The phytase enzyme works to catalyze the hydrolysis of phytate to inorganic phosphate (PO₄) and *myo*-inositol (Humer et al., 2015). While there is no requirement for *myo*-inositol, metabolically it is converted to glucose, and is a structural component of phosphoinositides, which regulate amylase secretion, insulin release, smooth muscle contraction, and liver glycogenolysis (McDowell, 2000). While feeding P above the pig's requirement may not improve growth performance, perhaps the additional *myo*-inositol release could help increase metabolic functions within the pig. However, because the pig can synthesize *myo*-inositol endogenously, it becomes difficult to determine whether its release from phytate has a beneficiary role (McDowell, 2000). The current study observed a tendency for an extra phosphoric effect when phytase was added to the positive control (formulated to meet the Ca and P requirements), with a tendency to improve G:F. Further research is needed to fully determine if Natuphos E does induce 'extra-phosphoric' effects and to confirm the benefit of additional *myo*-inositol release due to high concentrations of phytase, and its impact within the pig.

Overall, our study found growth performance improved as added dietary phytase increased up to 1,000 FTU/kg. Because pigs fed the NC + 500 FTU phytase and the PC did not

have similar growth performance or bone ash, we can conclude that the release value of 500 FTU/kg Natuphos E used in formulation overestimated the P release. A tendency for improved G:F was observed as phytase was added to the positive control diet when P and Ca were formulated at commercial industry levels. Lastly, the addition of phytase continued to increase percentage bone ash in the NC and when added to the PC, although there was little improvement in growth performance.

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Table 2-1 Analyzed ingredient composition (as-fed basis)¹

Ingredient	Analyzed value, %	
	P	Ca
Corn	0.31	0.03
Soybean meal	0.72	0.43
Limestone	0.23	37.73
Monocalcium P	20.54	16.38
Fish meal	3.07	5.59
Dried whey	0.80	0.58
Blood plasma	1.00	0.19
HP 300 ²	0.74	0.38
Corn DDGS, > 6 and < 9% oil	0.98	0.06
Trace mineral premix	0.03	18.28
Vitamin premix	0.04	18.17

¹Duplicate ingredient samples were pooled and analysis was performed at a commercial laboratory (Ward Laboratory; Kearney, NE).

²Hamlet Protein Inc. (Findlay, OH).

Table 2-2 Composition of basal batch (as-fed basis)¹

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	36.80	52.09	62.98
Soybean meal, 48% CP	20.80	27.46	32.93
Dairylac 80 ²	15.14	5.05	---
Dried whey	8.08	5.05	---
HP 300 ³	5.05	5.05	---
Corn DDGS	5.05	---	---
Blood plasma	4.04	---	---
Fish meal	1.26	1.26	---
Choice white grease	1.01	1.01	1.01
Monocalcium P	0.28	0.56	0.86
Limestone	1.19	0.98	0.83
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.30	0.38	0.35
DL-Met	0.17	0.20	0.14
L-Thr	0.12	0.16	0.13
L-Val	---	0.05	---
Trace mineral premix ⁴	0.15	0.15	0.15
Vitamin premix ⁵	0.25	0.25	0.25
Choline chloride 60%	0.04	---	---
	100	100	100
Calculated analysis			
Standardized ileal digestibility (SID) AA, %			
Lys	1.40	1.35	1.25
Ile:Lys	58	60	61
Leu:Lys	122	118	125
Met:Lys	33	37	34
Met & Cys:Lys	57	58	56
Thr:Lys	63	63	62
Trp:Lys	19.3	17.8	18.0
Val:Lys	68	69	66
Total Lys, %	1.58	1.50	1.39
CP, %	22.7	22.2	21.8
ME, kcal/kg	3,452	3,400	3,347
NE, kcal/kg	2,556	2,516	2,475
SID Lys:ME, g/Mcal	4.12	4.03	3.79
Ca, %	0.71	0.66	0.56
P, %	0.66	0.62	0.60
Available P ⁶ , %	0.40	0.30	0.25
STTD P, %	0.44	0.36	0.32

¹The basal batch was used as the major ingredient within each experimental diet. Treatment specific ingredients were added to the basal batch to achieve final dietary treatments.

²International Ingredient Corporation (St. Louis, MO).

³Hamlet Protein Inc. (Findlay, OH).

⁴Provided per kilogram of premix: 26.5 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc sulphate, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

⁵Provided per kg premix: 4,409,171 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 15 mg vitamin B12; 1,764 mg menadione; 3,307 mg riboflavin; 11,023 mg pantothenic acid, 19,841 mg niacin.

⁶Coefficients for formulation were derived from NRC (1998).

Table 2-3 Ingredient composition of experimental diets (as-fed basis)¹

Ingredient, %	Phase 1		Phase 2		Phase 3	
	Negative control	Positive control	Negative control	Positive control	Negative control	Positive control
Basal mix	96.52	96.52	98.43	98.43	98.75	98.75
Corn	3.35	2.52	1.46	0.63	1.10	0.25
Soybean meal	0.02	0.03	0.01	0.07	---	0.05
Limestone	---	0.73	---	0.08	---	0.08
Monocalcium P	---	0.05	---	0.70	---	0.75
Sand ¹	0.10	0.15	0.10	0.10	0.15	0.13
Phytase ²	---	---	---	---	---	---
Calculated analysis						
CP, %	22.8	22.8	22.2	22.2	21.2	21.3
Ca, %	0.71	0.85	0.66	0.80	0.56	0.70
P, %	0.66	0.81	0.63	0.77	0.61	0.76
Ca:P ratio	1.07	1.05	1.05	1.04	0.93	0.92

¹Sand was used to displace phytase in the diet as inclusion rate varied; as a result, the same amount of basal mix in each phase was added to each of the treatment diets.

²Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) was added to the negative control to achieve experimental diets with 0, 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg or was added to positive control diet to achieve experimental diets with 0 or 2,000 FTU/kg. Phytase inclusion was determined using the analyzed concentration, and the phytase contained 5,111,000 FTU/kg.

Table 2-4 Analyzed composition of experimental diets (as-fed basis)¹

Diets	Analyzed composition				
	CP, %	Ca, %	P, %	Ca:P	Phytase, FTU/kg
Phase 1					
NC ²	21.8	0.88	0.61	1.44	< 60
NC + 500 FTU	22.3	0.87	0.64	1.36	612
NC + 1,000 FTU	22.1	0.89	0.63	1.41	1,100
NC + 2,000 FTU	22.1	0.90	0.64	1.40	2,060
NC + 3,000 FTU	22.4	0.93	0.64	1.45	3,880
NC + 4,000 FTU	22.2	0.85	0.60	1.42	5,270
PC ³	21.8	1.10	0.76	1.45	< 60
PC + 2,000 FTU	22.4	1.07	0.80	1.34	2,580
Phase 2					
NC	21.8	0.75	0.59	1.27	< 60
NC + 500 FTU	21.6	0.78	0.58	1.34	650
NC + 1,000 FTU	21.3	0.83	0.61	1.36	1,350
NC + 2,000 FTU	21.9	0.84	0.63	1.33	2,590
NC + 3,000 FTU	22.6	0.75	0.56	1.33	3,630
NC + 4,000 FTU	22.6	0.89	0.67	1.33	5,200
PC	21.6	1.01	0.74	1.36	< 60
PC + 2,000 FTU	22.2	0.94	0.75	1.25	2,560
Phase 3					
NC	20.8	0.75	0.63	1.19	< 60
NC + 500 FTU	22.0	0.75	0.61	1.23	536
NC + 1,000 FTU	21.6	0.73	0.60	1.22	1,190
NC + 2,000 FTU	21.5	0.78	0.61	1.28	2,280
NC + 3,000 FTU	21.9	0.70	0.60	1.17	3,710
NC + 4,000 FTU	21.8	0.70	0.63	1.11	4,660
PC	21.9	0.87	0.77	1.13	62
PC + 2,000 FTU	22.2	0.87	0.77	1.13	2,110

¹Seven subsamples were pooled and proximate analysis was performed in triplicate by a commercial laboratory (Ward Laboratories, Kearney, NE). In addition, phytase analysis was conducted in duplicate to determine complete diet phytase concentrations at another commercial laboratory (Eurofins Scientific Inc., Des Moines, IA).

²Negative Control.

³Positive Control.

Table 2-5 Effect of high doses of Natuphos E 5,000 G on nursery pig growth performance and bone ash values¹

Item	Negative Control ²							Positive Control ³		SEM	<i>P</i> <				
	0	500	1,000	2,000	3,000	4,000	0	2,000	Negative Control		NC vs. PC	NC + 500 vs. PC ⁴	PC vs. PC + 2,000		
									Linear					Quadratic	
BW, kg															
d 0	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	0.01	0.250	0.818	0.687	0.230	0.421	
d 42	21.5	21.4	22.6	22.2	22.5	22.2	22.7	22.6	0.38	0.082	0.128	0.035	0.021	0.921	
d 0 to 7															
ADG, g	64	74	84	72	77	83	83	75	9.6	0.293	0.785	0.145	0.475	0.512	
ADFI, g	112	114	124	108	118	129	123	115	6.9	0.165	0.347	0.248	0.332	0.403	
G:F, g/kg	562	645	675	666	636	640	665	616	57.1	0.558	0.228	0.157	0.785	0.499	
d 7 to 21															
ADG, g	276	271	297	277	295	291	295	285	14.9	0.274	0.763	0.333	0.215	0.630	
ADFI, g	349	345	363	352	361	362	369	349	13.9	0.343	0.851	0.250	0.173	0.241	
G:F, g/kg	791	782	816	786	814	802	795	818	18.1	0.456	0.774	0.866	0.595	0.352	
d 21 to 42															
ADG, g	541	541	569	569	568	557	577	581	13.5	0.192	0.078	0.038	0.048	0.847	
ADFI, g	820	790	844	837	838	847	871	849	19.6	0.095	0.644	0.049	0.003	0.398	
G:F, g/kg	659	685	674	680	678	658	663	685	7.8	0.493	0.001	0.696	0.047	0.047	
d 0 to 42															
ADG, g	369	377	396	387	395	381	400	398	10.2	0.314	0.064	0.027	0.107	0.864	
ADFI, g	540	529	562	551	559	555	580	561	13.5	0.188	0.427	0.029	0.007	0.289	
G:F, g/kg	684	713	705	702	707	686	689	709	7.8	0.616	0.008	0.571	0.032	0.060	
Bone ash, g ⁵	1.94	2.30	2.35	2.56	2.53	2.25	2.42	2.51	0.093	0.012	0.001	0.001	0.374	0.465	
Bone ash, %	44.2	45.2	47.1	48.0	48.4	49.1	47.0	51.3	0.007	0.001	0.078	0.010	0.099	0.001	

¹A total of 360 barrows (DNA 200 × 400, initially 5.9 ± 0.1 kg) were used in a 42-d growth study with 5 pigs per pen and 9 pens per treatment (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ).

²Negative control diets were formulated with 0.40, 0.30, or 0.25% aP from inorganic P for Phases 1, 2 and 3 respectively. Phytase was added at 0, 500, 1,000, 2,000, 3,000, 4,000 FTU/kg to the negative control diet to achieve final experimental diets.

³Positive control diets were formulated with 0.55, 0.45, or 0.40% aP from inorganic P for Phases 1, 2 and 3 respectively. Phytase was added at either 0 or 2,000 FTU/kg to the positive control diet to achieve final experimental diets.

⁴NC diets were formulated to be the PC minus 0.15% P and 0.14% Ca released by 500 FTU/kg Natuphos E suggested by the manufacturer. The NC + 500 FTU and PC treatments were compared to confirm the estimated release value.

⁵One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

Chapter 3 - Determining the impact of increasing standardized ileal digestible lysine for primiparous and multiparous sows in lactation

Abstract

Two experiments were conducted to evaluate the effects of increasing dietary SID Lys in lactation on sow and litter performance. In Exp. 1, 111 primiparous sows (Line 241; DNA, Columbus, NE) were allotted to 1 of 4 dietary treatments on d 110 of gestation. Dietary treatments included increasing dietary standardized ileal digestible (SID) Lys (0.80, 0.95, 1.10, and 1.25%). During lactation, there were no differences in ADFI or sow BW at weaning (d 21), resulting in no differences in BW loss. However, backfat loss during lactation decreased (linear, $P = 0.046$) as SID Lys increased. There were no differences in litter weaning weight, litter gain from d 2 to weaning, percentage of females bred by d 7 after weaning, d 30 conception rate, farrowing rate or subsequent litter characteristics. In Exp. 2, 710 mixed parity sows (Line 241; DNA, Columbus, NE) were allotted to 1 of 4 dietary treatments at d 112 of gestation. Dietary treatments included increasing SID Lys (0.75, 0.90, 1.05, and 1.20%). Sow BW at weaning increased (quadratic, $P = 0.046$), and sow BW loss from post-farrow to weaning or d 112 to weaning decreased (quadratic, $P \leq 0.01$) as SID Lys increased. Sow backfat loss increased (linear, $P = 0.028$) as SID Lys increased. Conversely, longissimus muscle depth loss decreased (linear, $P = 0.002$) as SID Lys increased. Percentage of females bred by d 7 after weaning increased (linear, $P = 0.047$) as SID Lys increased in parity 1 sows, with no difference in parity 2 or 3+ sows. Litter weight at d 17 and litter gain from d 2 to 17 increased (quadratic, $P = 0.01$) up to 1.05% SID Lys with no improvement thereafter. For subsequent litter characteristics, there were no differences in total born, percentage born alive, stillborn or mummies. In conclusion, our results suggest that increasing dietary SID Lys can reduce sow protein loss in lactation. The

optimal level of dietary SID Lys required by the sow may vary based on response criteria and parity.

Key words: Gilt, lactation, lysine, reproduction, sow

Introduction

Over the past two decades, genetic improvements have increased the efficiency and productivity of the sow herd. As genetics continue to evolve, requirement estimates need to be reevaluated to ensure all nutrients are met for optimum performance. In lactation, nutrients need to be supplied to support both sow maintenance and litter growth (Dourmad et al., 2008). With milk production representing about 75% of total nutrient requirements in lactation (Noblet et al., 1990), it becomes increasingly more difficult to meet the sow's requirements as litter size increases without also changing diet formulation.

Inadequate nutrient intake during lactation can cause the sow to become catabolic and increase sow body protein mobilization (Yang et al., 2000). Increases in body weight loss from reduced or restricted nutrient intake, and resulting body protein mobilization during lactation can decrease the subsequent litter size due to reduced follicular development (Clowes et al., 2003) or embryonic survival (Vinsky et al., 2006). However, recent research has demonstrated that commercial primiparous sows may be more resistant to negative effects of lactational catabolism from reduced feed intake (Patterson et al., 2011).

Lysine is the first limiting amino acid in corn and soybean meal-based swine diets. Because primiparous sows consume less feed than multiparous sows (Koketsu et al., 1996), maternal growth accounts for a larger percentage of daily nutrient intake. Early literature has shown decreased BW loss but no difference in litter performance when Lys increased from 0.67 to 1.0% in the diet of lactating gilts (Boomgaard et al., 1972; Dourmad et al., 1998), with varying results in sows depending on response criteria (Boyd et al. 2000). However, with modern

genetics and greater productivity levels, requirements to reduce mobilization of body protein reserves, maximize litter growth, and maintain reproductive function in high producing multiparous sows needs to be re-evaluated. Therefore, the objective of these experiments was to determine the effect of increasing standardized ileal digestible (SID) Lys on the performance of 1) lactating primiparous sows and their litters, and 2) mixed parity sows and their litters under commercial conditions.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments.

Experiment 1

A total of 111 primiparous sows (Line 241, DNA, Columbus, NE) were used over 4 consecutive farrowing groups. The trial was conducted at the Kansas State University Teaching and Research Center in Manhattan, KS from January to April, 2016. At d 110 of gestation, sows were weighed and moved to the farrowing house. Females were blocked by weight and expected farrowing date and randomly allotted to 1 of 4 treatments within those blocks. Dietary treatments were corn-soybean meal-based and consisted of increasing SID Lys (0.80, 0.95, 1.10, or 1.25%). Treatments were formed by increasing both crystalline Lys and soybean meal such that there was an increase in L-Lys HCl of 0.12% between each treatment with soybean meal increasing to meet the remainder of the SID Lys target for each treatment. Other feed-grade AA were added as needed to maintain a similar minimum ratio to Lys. All other nutrients met or exceeded the NRC (2012) requirement estimates (Table 3-1).

From d 110 to 113 of gestation, sows were fed 2.7 kg/d of the gestation diet (14.1 % CP, 0.56% SID Lys, 1,472 ME/kg). Starting on d 113, sows received 2.7 kg/d of dietary treatment until farrowing. Postpartum, sows were allowed ad libitum access to feed, recorded by weighing

the amount placed in a feed hopper and the amount remaining at weaning (d 21 ± 3). Sow BW and back fat depth (measured 10 cm from the midline of the last rib) were measured on d 0, d 10 post-farrowing, and at weaning. Cross fostering occurred irrespective of dietary treatment until 48 h postpartum in an attempt to equalize litter size (minimum of 10 pigs per litter for group 1 and 12 pigs per litter for groups 2 to 4). Litters were weighed on d 2 and 10 post-farrowing and at weaning.

At weaning (average of 18.7 d post farrowing and range of 15 to 23), sows were moved to a breeding barn, housed individually, and checked daily for signs of estrus using a boar. The wean-to-estrus interval (WEI) was determined as the number of days between weaning and when sows were first observed to show a positive response to the back-pressure test. Conception rate was determined based on confirmation of pregnancy by ultrasound test at approximately d 30 post breeding.

After weaning, no dietary treatments were applied, and females were fed a common gestation diet with 0.56% SID Lys according to their body condition. Thin, ideal, and fat sows were fed 2.1, 2.0, or 1.9 kg/d, respectively. Subsequent performance (total born, number born alive, stillborn and mummies) was collected from sows on the subsequent farrowing. Diets were manufactured at the Kansas State University O.H. Kruse Feed Mill in Manhattan, KS. A new batch of each treatment diet was manufactured for each farrowing group and packaged in 22 kg bags. During bagging, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, 30th, and 35th bag, and these samples were pooled and used for AA and nutrient analysis.

Chemical analysis

Four samples (one per batch) per dietary treatment from the pooled samples were sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP (AOAC 900.03, 2006), Ca (AOAC 965.14/985.01, 2006), and P analysis (AOAC 965.17/985.01,2006). In addition, 4

samples (one per batch) per dietary treatment were sent to another commercial laboratory (Ajinomoto Heartland Inc., Eddyville, IA) for complete diet amino acid analysis (AOAC 994.12, Table 3-3).

Data analysis

Data were analyzed using generalized linear mixed models where dietary treatment was a fixed effect, with random effects of group and block. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC).

Sow ADFI, BW, BW change, backfat change, litter weight, litter gain, lactation length, and SID Lys consumed were fitted assuming a normal distribution of the response variable. Litter weight and litter count on d 2 were used as covariates for d 10 litter weight, weaning litter weight and litter weight gain. In these cases, residual assumptions were checked using Studentized residuals and were found to be reasonably met.

Wean-to-estrus interval, litter size and subsequent total born were fitted assuming negative binomial distribution. Females bred until d 7 after weaning, d 30 conception rate and farrowing rate were fitted using a binary distribution. Subsequent litter performance variables, born alive, percentage stillborns and mummies, were all fitted using a binomial distribution. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Experiment 2

The experiment was conducted on a commercial sow farm in central Nebraska from mid-June until mid-August, 2016. Females were individually housed from d 0 to d 113 of gestation and were fed a common diet with 0.70% standardized ileal digestible (SID) Lys according to body condition (thin, ideal, and fat females were offered 2.5, 1.8 and 1.3 kg, respectively). All females had ad libitum access to water.

A total of 710 primiparous and multiparous females (Line 241, DNA Genetics, Columbus, NE) were used. At d 112 of gestation, females were weighed, and on a subsample of females (n = 369), back fat and longissimus muscle depth were collected via ultrasound (Aloka SSD 500V, Hitachi Aloka Medical Ltd., Wallingford, CT; between 10th and 11th ribs, 2.5 cm from the midline). Females were blocked by BW within expected farrowing date and parity (1 to 7) and were then randomly assigned to 1 of 4 dietary treatments within blocks. Dietary treatments were corn-soybean meal-based and consisted of increasing SID Lys (0.75, 0.90, 1.05 and 1.20%). Treatments were formulated like in Exp. 1 by increasing both crystalline Lys and soybean meal to maintain a similar soybean meal to crystalline Lys ratio. Other feed-grade AA were added as needed to maintain a similar minimum ratio to Lys across treatments. All other nutrients met or exceeded the NRC (2012) requirement estimates. Energy (ME, kcal/kg) was the same across all dietary treatments (Table 3-2).

On d 113 of gestation, females were moved to the farrowing house and fed treatment diets. Sows received 2.5 kg/d of feed until farrowing. Cross fostering occurred irrespective of dietary treatment until 48 h postpartum in an attempt to equalize litter size (minimum of 10 pigs per litter). Litters were weighed on d 2 (after equalization) and d 17 post-farrowing.

At weaning (average of 21.3 d post farrowing and range of 19 to 24) sows were returned to the gestation barn where sow body weight was determined and back fat and longissimus muscle depth were again measured via ultrasound. Each sow was housed individually and checked daily for signs of estrus using a boar. The WEI was determined as the number of days between weaning and when sows were first observed to show a positive response to the back-pressure test. Conception rate was determined based on pregnancy confirmation using ultrasound at approximately d 30 after first insemination.

Due to the magnitude of this study and the commercial setting, sow BW 24 h after farrowing was not able to be measured. A post-farrowing weight was calculated for each sow by subtracting the weight of conceptus from each sow's d 112 body weight. Final weight of conceptus was calculated using the original equation listed in the NRC (2012) generated from Dourmad et al. (1998), and corrected by Thomas et al. (2016) using the variables of parity, length of gestation and total born.

No dietary treatments were applied after weaning and all females were fed a blend of 1.8 kg of a 0.70% SID Lys gestation diet and 1.3 kg of a 1.05% SID Lys lactation diet until breeding. After breeding, each sow was fed the gestation diet according to their body condition for the remainder of gestation (thin, ideal, and fat females were fed 2.5, 1.8 and 1.3 kg respectively). Subsequent performance (total born, number born alive, mummies and stillborn) were collected from sows remaining in the herd on their subsequent parity.

Experimental diets were manufactured at the Pillen Family Farms Feed Mill (Albion, NE). Feed was continuously delivered in bulk throughout the study period, and feed delivery amounts by treatment were recorded to determine total feed consumed in lactation. Average daily feed intake by treatment was calculated by total feed delivered during the trial period divided by number of sows on each treatment diet for each day of the trial period.

Chemical analysis

Samples of the diet were taken at the feeder, three times per week. Samples were pooled by week to make a composite sample. Six samples per dietary treatment were sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP (AOAC 900.03, 2006), Ca (AOAC 965.14/985.01, 2006), and P (AOAC 965.17/985.01, 2006). Additionally, 6 samples (one per week) per dietary treatment were sent to another commercial laboratory (University of

Missouri Experimental Station Chemical Laboratories, Columbia, MO) for complete diet amino acid analysis (AOAC 975.44 and 982.30, Table 3-4).

Data analysis

Data were analyzed using generalized linear mixed models where dietary treatment and parity category (P1, P2, and P3+) and dietary treatment within parity category were evaluated as fixed effects, with random effect of block. The response variables of sow BW (d112, post farrow, and weaning), BW loss, backfat change, longissimus muscle depth change, litter weight (d 2 and d 17), litter gain and lactation length were fitted assuming a normal distribution. Total born was used as a covariate for post farrow sow BW. Longissimus muscle depth on d 112 was used as a covariate for its depth at weaning and change over lactation. Litter weight on d 2 was used as a covariate to improve the fit of the model for d 17 litter weight, and litter gain response variables. Litter weight on d 2 and lactation length were used as covariates for sow weaning BW, sow BW change from d 112 to weaning, and sow BW change from post farrow to weaning.

Day 2 litter size, d 17 litter size and subsequent total born were fitted using a negative binomial distribution. Females bred by d 7, d 30 conception rate and farrowing rate were fitted using a binary distribution. Subsequent born alive, stillborn, and mummies were modeled using a binomial distribution.

Results were considered significant at $P \leq 0.05$. Use of covariates were included in the model if they improved the Bayesian information criterion (BIC) by greater than 2 units. For normally distributed data the residual assumptions were found to be reasonably met using evaluation of the Studentized residuals. Statistical models were fit using PROC GLIMMIX of SAS (Version 9.4, SAS Institute Inc., Cary, NC).

Results and Discussion

Chemical analysis

In Exp. 1, chemical analysis of DM, CP, Ca, P, and AA were similar to the formulated values (Table 3-3). The analyzed total Lys concentration in the 0.80% SID Lys diet was slightly higher than formulated. In Exp. 2, chemical analysis of DM, CP, Ca, P, and AA were similar to the formulated values (Table 3-4).

Sow BW, backfat and loin eye depth change in lactation

There were no differences among treatments in initial BW or backfat depth in Exp. 1 (Table 3-5) and Exp. 2 (Table 3-7) which validated treatment randomization. In Exp. 2, increasing SID Lys to 1.20% reduced BW loss within parity 2 (linear, $P = 0.028$) and parity 3 + (quadratic, $P < 0.007$) sow categories (Figure 3-1). Sow BW loss in lactation is inevitable due to higher nutrient demands than voluntary feed intake can support. Previous studies (Dourmad et al., 1998; Xue et al., 2012; Huang et al., 2013) found a decrease in BW loss as Lys increased in the diet. The results of multiparous sows do not agree with the results of primiparous sows where no differences in BW loss regardless of dietary Lys concentration in Exp. 1 ($P = 0.235$) and Exp. 2 ($P = 0.361$) were found. In support of these findings, Yang et al. (2000) and Xue et al. (2012) did not observe any differences in BW loss as Lys increased in first parity sows. However, Shi et al. (2015) observed that primiparous sow BW loss decreased with increasing SID Lys and estimated the optimal dietary SID Lys for minimal BW loss at 0.85%. The summary of research and present data would suggest that BW loss can be reduced when increasing SID Lys in multiparous sows, with minor or no benefit in primiparous sows.

Previous literature observed no differences in backfat loss during lactation regardless of dietary SID Lys concentration (Touchette et al., 1998; Yang et al., 2000; Shi et al., 2015). In Exp. 1 increasing dietary SID Lys decreased (linear, $P = 0.046$) backfat loss in first parity sows.

Conversely, in Exp. 2 backfat loss increased (linear, $P = 0.028$) with increasing SID Lys. One explanation for this may be that the increase in litter growth rate as SID Lys increased would require more energy and if feed intake did not differ, mobilization of lipid stores to satisfy milk production would be required (Dourmad et al., 2008). However, sows fed 1.20% SID Lys had decreased litter growth, which may have been due to a reduction in ADFI and thus energy intake. The decrease in energy intake could have contributed to additional back fat loss on the 1.20% SID Lys treatment.

In Exp. 2, as SID Lys increased in the diet we observed a reduction in loin eye depth loss during lactation (linear, $P = 0.002$) from -1.9 to 0.5 mm, which resulted in an increase (linear, $P = 0.002$) in actual loin eye depth at weaning. In support of these findings, Shi et al. (2015) observed a quadratic decrease in loin eye area loss during lactation with increasing SID Lys, with the greatest reduction occurring at 1.02% SID Lys (54 g/d total Lys) in primiparous sows. In addition, Touchette et al. (1998) determined that minimum loin eye area loss occurred at 48 g/d SID Lys, and Dourmad et al. (1998) observed losses in lean tissue were greater in sows fed 0.67% total Lys compared to 0.77 to 0.87% total Lys. It is likely that body protein mobilization occurs when the sow is deficient in AA intake, but is not necessarily independent from an energy deficiency (Dourmad et al., 2008). When energy intake is insufficient, a sow may mobilize body protein to support the energy deficiency (Pomar et al., 1991). When evaluating restricted total dietary Lys intake, Clowes et al. (2003) looked at the amount of protein loss that could be sustained by a lactating sow without impacting performance. Their study demonstrated that there were no differences in body protein loss up to d 20 of lactation; however, from d 20 to weaning on d 23, there was significant body weight loss, which followed an increase in body protein loss. This suggests that until d 20 of lactation, a minimal amount of body reserves have been mobilized even when severely deficient in total dietary Lys, but after d 20 as milking

pressure and litter growth increases, larger amounts of body protein mobilization occur, and dietary Lys is needed to reduce negative impacts from excessive protein mobilization.

Sow ADFI

In Exp. 1, no difference ($P = 0.821$) was observed in ADFI as dietary SID Lys increased from 0.80 to 1.25%. Multiple studies (Huang et al., 2013; Huber et al., 2015; Shi et al., 2015) have shown no differences in ADFI with increasing SID Lys from 0.76 to 1.14%. One study observed a decrease in ADFI as total Lys increased from 0.60 to 1.60% (Yang et al., 2000), and hypothesized the decrease in intake was due to elevated serum urea nitrogen levels and varying branch chain AA ratios across their experimental diets. Because our SID Lys range falls within the range of Yang et al. (2000), it is not clear whether SID Lys levels above 1.25% deter feed intake. In Exp. 2, we were unable to capture individual sow ADFI and cannot make a conclusion on the effect of dietary SID Lys on individual sow intake in that study. However, ADFI for each treatment was calculated using the total amount of feed delivered for each treatment divided by total sows consuming each diet. Average daily feed intake increased as SID Lys increased from 0.75 to 1.05%, but ADFI decreased on the 1.20% SID Lys treatment.

Litter performance

There were no differences in lactation length, litter size on d 2 or litter size at weaning in Exp. 1 and Exp. 2. In addition, in Exp. 1 regardless of treatment, there were no differences in litter weight at d 2 (Table 3-6). However, in Exp. 2 litter weight at d 2 increased from 21.2 to 22.2 kg (quadratic, $P = 0.016$) as SID Lys level increased up to 1.05% SID Lys, this was unexpected due to cross fostering to equalize litter size.

An early study by Johnston et al. (1993) suggests a linear correlation exists between Lys intake in lactation and litter weight gain. More recently, Xue et al. (2012) observed a linear increase in litter weight gain as SID Lys increased from 45.0 to 68.5 g/d in mixed parity sows.

Yang et al. (2000) observed a quadratic response in litter gain with optimal litter growth rates occurring at 44, 55 and 56 g/d total Lys for parities 1, 2, and 3 respectively. These two studies are in agreement with Exp. 2 where litter gain increased (quadratic, $P = 0.001$) with increasing SID Lys and was maximized at 1.05% SID Lys for mixed parity sows. There was a decrease observed for litter growth in sows fed 1.20% SID Lys, however there was also a decrease in ADFI on this treatment. Tokach et al. (1992) observed that energy had to be increased along with Lys in order to obtain benefits in milk output, which would suggest why we saw a decrease in litter growth on the highest Lys treatment with reduced feed intake.

In contrast, no differences in litter growth were observed by Dourmad et al. (1998), Shi et al. (2015) and Huber et al. (2015) when fed increasing dietary levels of 0.66 to 0.87%, 0.76 to 1.14%, or 0.73 to 0.94% SID Lys, respectively. In Exp. 1, no improvement ($P = 0.209$) in litter gain was observed as SID Lys increased from 39 to 63 g/d for primiparous sows. However, the lowest SID Lys fed in Exp. 1 would be near the SID Lys of 44 g/d for optimal litter growth rate observed by Yang et al. (2000) in parity 1 sows, which could be why no difference was observed in our study. When calculating the estimated SID Lys (g/d) per kg of litter gain in Exp. 1, the 0.80 and 0.95% SID Lys treatments were supplying 39.9 and 45.0 g/d of SID Lys, respectively. This is less than the predicted requirement of 47.4 to 48.7 g/d SID Lys that the NRC (2012) model would estimate for the observed litter growth for all treatments. However, no differences in litter growth were observed. This coupled with greater backfat loss on the low SID Lys treatments in Exp. 1 demonstrates that sows will continue to mobilize body reserves to meet the demands of milk production and litter growth when diets are low in SID Lys. When estimating the g/d SID Lys recommended per kg of litter growth observed in Exp. 2 (NRC, 2012), our sows should require 43 to 47 g SID Lys. However, our estimated consumption based on ADFI was 48 to 70 g/d of SID Lys. This would mean our sows in Exp. 2 were consuming more SID Lys than

what was needed for litter growth and may have been depositing excess SID Lys and AA's as body protein, which is supported by increased loin eye depth at weaning with increasing SID Lys. Our study would be in agreement with previous literature (Touchette et al., 1998) that suggests the Lys requirement for litter growth is less than that for reducing loin eye depth loss. Our study would also demonstrate that 39.9 g/d of SID Lys was sufficient to meet litter demands in primiparous sows. It is important to note that the NRC (2012) model for SID Lys requirement per kg of litter growth does not incorporate studies with litter growth greater than 2.7 kg per day. Thus, with the use of more recent genetic lines in the current study, we observed litter growth rates that were above those used to create the model, which may be why the calculated SID Lys requirements for litter growth observed in our study were over predicted.

Unlike multiparous sows who show an increase in milk yield when made anabolic during lactation, primiparous sows seem to partition extra energy into body growth rather than milk production (Pluske et al. 1998). This could explain why only multiparous sows showed an increase (quadratic, $P = 0.001$) in litter growth when supplied with additional SID Lys in lactation. Clowes et al. (2003) suggests that there is no impact in litter growth up to d 20 of lactation when total dietary Lys consumption ranged from 24 to 50 g/d, however from d 20 to weaning at d 23, there was significant decrease in litter growth and milk protein concentration due to large amounts (> 12%) of sow body protein being mobilized on the low total Lys treatment. This indicates that until d 20 of lactation, a minimal amount of body reserves has been mobilized but as the milking pressure and litter growth increases after that point, increasing dietary SID Lys and other AA's may be needed to maintain productivity.

Reproductive performance

In Exp. 1, there was no difference ($P = 0.975$) in percentage of females bred by d 7 after weaning. This is in agreement with Yang et al. (2000) and Shi et al. (2015) where no differences

were found in WEI or percentage mated post weaning in primiparous sows. However, in the commercial setting of Exp. 2, there was a difference in percentage of females bred by 7 d in parity 1 sows, where increasing SID Lys from 0.75 to 1.20% increased (linear, $P = 0.047$) the percentage of females mated (Figure 3-2). Similarly, Xue et al. (2012) observed a decrease in WEI as SID Lys increased. These conflicting results could be due to the research setting (commercial vs. university) and make it difficult to determine the optimal SID Lys level in first parity sows needed to minimize WEI and improve the percentage of females bred by d 7 after weaning.

In Exp. 2, no difference was observed in percentage of parity 2 or parity 3+ sows bred by d 7 after weaning which is in agreement with results of Yang et al. (2000). However, as SID Lys increased in parity 1 sows, protein loss was reduced and there was an increase in percentage bred by d 7 after weaning. This would suggest that increased body protein mobilization in parity 1 sows decreases signs of estrus by d 7 after weaning. Similarly, King (1987) observed a shorter wean to estrus interval when body protein loss was minimized in parity 1 sows. Furthermore, because expression of estrus by d 7 increased in parity 1 sows as SID Lys increased to 1.20%, and litter growth was maximized at 1.05% SID Lys, it can be hypothesized that the SID Lys requirement for reproduction is greater than that for litter growth.

There were no differences observed in d 30 conception rate in Exp. 1 or Exp. 2. Few studies report a value for conception rate; however, Shi et al. (2015) observed no differences in conception rate as SID Lys increased from 0.76 to 1.14%. No difference in farrowing rate was observed in either of our studies, which is in agreement with the current body of literature (Touchette et al., 1998; Yang et al., 2000).

Subsequent litter characteristics

A study conducted by Yang et al. (2000) observed a decrease in total born and born alive and increased stillborns for the subsequent litter as SID Lys level in the previous lactation increased. However, they explained that litter size (total born and born alive) did not differ in sows fed 0.60 to 1.10% SID Lys and was only reduced in sows fed 1.35 and 1.60%. They hypothesized this decrease at the highest SID Lys concentrations was due to elevated serum urea nitrogen levels or low lactation feed intake. Touchette et al. (1998) also saw decreased total born and born alive with increasing dietary SID Lys in lactation, but only when ratios of other amino acids to SID Lys were held constant, thus increasing as SID Lys increased. They suggest that litter size may be affected by different amino acid ratios in the diet. Clowes et al. (2003) demonstrated that protein restriction during lactation can negatively affect follicle size and, consequently, ovulation rate, which may reduce subsequent total born.

In contrast, both of our studies demonstrated that there were no differences in subsequent total born, born alive, stillborn or mummies as SID Lys level increased in the previous lactation. More recently, Shi et al. (2015) observed no difference in subsequent total born, born alive, or stillborn when SID Lys was increased from 0.76 to 1.14%. Schenkel et al. (2010) conclude that subsequent litter size is affected by absolute body reserves at weaning and the amount of tissue mobilization during lactation. Their study does not mention any data on percentage of piglets born alive, stillborn or mummies. In addition, tissue mobilization in the current studies did not occur at the same level as described by Schenkel et al. (2010), and may suggest that we minimized any reduction in subsequent total born with the levels of SID Lys fed.

In conclusion, our results demonstrate that the sow will mobilize body fat reserves to satisfy litter growth requirements if nutrients are not met by dietary intake. However, increasing the levels of AA's can support the reduction of protein loss in lactation. While the optimal level

of dietary SID Lys required by the sow may vary based on response criteria and parity, it is evident that reducing protein mobilization is beneficial to reproductive performance.

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Table 3-1 Diet composition (as-fed basis), Exp. 1¹

Ingredient, %	Standardized ileal digestible Lys, %			
	0.80	0.95	1.10	1.25
Corn	68.17	65.64	63.00	60.38
Soybean meal, 46.5% CP	25.58	27.89	30.21	32.49
Choice white grease	2.00	2.00	2.00	2.00
Limestone	1.30	1.28	1.28	1.25
Monocalcium P, 21% P	1.80	1.78	1.75	1.75
Salt	0.50	0.50	0.50	0.50
L-Lys-HCl	---	0.12	0.24	0.36
DL-Met	---	0.01	0.07	0.14
L-Thr	---	0.06	0.13	0.20
L-Trp	---	---	---	0.02
L-Val	---	0.09	0.18	0.28
Trace mineral premix ²	0.15	0.15	0.15	0.15
Sow vitamin premix ³	0.25	0.25	0.25	0.25
Vitamin premix ⁴	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	0.80	0.95	1.10	1.25
Ile:Lys	80	72	65	61
Leu:Lys	173	151	135	123
Met:Lys	32	29	31	34
Met & Cys:Lys	63	56	56	56
Thr:Lys	69	67	67	67
Trp:Lys	23	21	19	19
Val:Lys	89	87	87	87
Total Lys, %	0.93	1.08	1.24	1.40
ME, kcal/kg	3,313	3,315	3,318	3,322
CP, %	17.8	18.9	20.1	21.3
Ca, %	0.90	0.90	0.90	0.90
P, %	0.75	0.75	0.75	0.75
Available P, %	0.45	0.45	0.45	0.45

¹Diets were fed from d113 of gestation to weaning.

²Provided per kilogram of premix: 26.5 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc sulfate, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

³Provided per kg premix: 4,409 IU vitamin A; 44 mg biotin; 992 mg vitamin B6; 331 mg folic acid; 110,229 mg choline; 9,921 mg L-carnitine.

⁴Provided per kg premix: 4,409,171 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 15 mg vitamin B12; 1,764 mg menadione; 3,307 mg riboflavin; 11,023 mg pantothenic acid, 19,841 mg niacin.

Table 3-2 Diet composition (as-fed basis), Exp. 2¹

Ingredient	Standardized ileal digestible Lys, %			
	0.75	0.90	1.05	1.20
Corn	73.40	68.36	63.28	58.51
Soybean meal, 46.5% CP	19.28	24.23	29.18	33.96
Corn oil	3.00	3.00	3.00	3.00
Limestone	1.41	1.39	1.36	1.34
Monocalcium P, 21%	1.33	1.30	1.27	1.24
Salt	0.50	0.50	0.50	0.50
L-Lys-HCL	0.15	0.19	0.23	0.28
L-Thr	0.04	0.07	0.11	0.15
L-Trp	0.01	0.01	0.02	0.02
DL-Met	---	0.003	0.05	0.09
L-Val	0.06	0.12	0.18	0.24
Sal Curb ²	0.33	0.33	0.33	0.33
Sow vitamin/mineral premix ³	0.20	0.20	0.20	0.20
Choline chloride	0.13	0.13	0.13	0.13
AxtraPhy 2500 ⁴	0.02	0.02	0.02	0.02
Dye ⁵	0.16	0.16	0.16	---
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	0.75	0.90	1.05	1.20
Ile:Lys	71	68	66	64
Met: Lys	30	30	30	31
Met & Cys: Lys	61	56	56	56
Thr:Lys	67	67	67	67
Trp:Lys	20	20	20	20
Val:Lys	90	90	90	90
Total Lys, %	0.87	1.04	1.20	1.37
ME, kcal/kg	3,479	3,479	3,479	3,479
CP, %	15.5	17.5	19.6	21.6
Ca, %	0.85	0.85	0.85	0.85
P, %	0.62	0.63	0.65	0.66
Available P, %	0.45	0.45	0.45	0.45

¹Diets were fed from d 114 of gestation to weaning.

²Kemin Industries (Des Moines, IA)

³Provided per kg of premix: 18 mg Cu; 0.8 mg I; 100 mg Fe; 40 mg Mn; 0.15 mg Se; 125 mg Zn; 11,000 IU vitamin A, 1,980 IU vitamin D; 99 IU vitamin E; 4 mg vitamin K; 0.04 mg vitamin B12; 44.2 mg niacin; 27.5 mg pantothenic acid; 8.6 mg riboflavin; 3.1 mg folic acid; 0.44 mg biotin; 5.1 mg vitamin B6; 2.2 mg thiamin; 0.44 mg chromium.

⁴Dupont (St. Louis, MO)

⁵Different colored dyes were added to distinguish among diets at the farm.

Table 3-3 Chemical analysis of diets (as-fed basis), Exp.1¹

Item, %	Standardized ileal digestible Lys, %			
	0.80	0.95	1.10	1.25
DM	88.32	88.14	88.29	88.37
CP	17.99	18.68	20.01	21.40
Ca	1.03	1.05	1.08	1.09
P	0.77	0.75	0.79	0.79
Total AA, %				
Lys	1.01	1.12	1.26	1.43
Ile	0.72	0.74	0.78	0.85
Leu	1.55	1.48	1.64	1.74
Met	0.30	0.31	0.37	0.44
Met & Cys	0.63	0.64	0.71	0.81
Thr	0.70	0.77	0.86	0.97
Trp	0.22	0.22	0.24	0.27
Val	0.85	0.92	1.06	1.21
His	0.48	0.49	0.50	0.54
Phe	0.84	0.93	0.97	1.03

¹Diet samples were collected from each batch of feed at manufacturing from every fifth bag. Crude protein and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc. (Chicago, IL). Dry matter, Ca, and P analyses were conducted on composite samples by Ward Laboratories (Kearney, NE).

Table 3-4 Chemical analysis of the diets (as-fed basis), Exp. 2¹

Item, %	Standardized ileal digestible Lys, %			
	0.75	0.90	1.05	1.20
DM	88.02	88.24	88.54	88.88
CP	14.78	16.87	18.25	20.08
Ca	0.97	0.94	1.04	0.99
P	0.62	0.66	0.62	0.63
Total AA, %				
Lys	0.89	1.03	1.19	1.31
Ile	0.64	0.71	0.82	0.88
Leu	1.36	1.48	1.61	1.69
Met	0.22	0.24	0.32	0.38
Met & Cys	0.46	0.50	0.62	0.68
Thr	0.58	0.68	0.79	0.89
Trp	0.12	0.15	0.16	0.18
Val	0.82	0.94	1.12	1.25
His	0.38	0.42	0.48	0.51
Phe	0.75	0.84	0.94	1.00
Free lys	0.12	0.14	0.18	0.21

¹Diets were collected twice per week and pooled to make a composite sample. Six composite samples per dietary treatment were sent for analysis. Total AA analyses were conducted on composite samples by University of Missouri Experimental Station Chemical Laboratories (Columbia, MO). Dry matter, Crude protein, Ca and P analyses were conducted by Ward Laboratories (Kearney, NE).

Table 3-5 Effects of increasing standardized ileal digestible (SID) lysine in lactation diets on sow performance, Exp. 1¹

Item	Standardized ileal digestible Lys, %				SEM	Probability, <i>P</i> <	
	0.80	0.95	1.10	1.25		Linear	Quadratic
BW, kg							
d 110	194.9	196.0	195.2	195.7	2.97	0.929	0.894
d 0	184.3	184.0	183.7	185.2	2.50	0.835	0.667
d 10	182.3	181.8	182.8	183.9	2.43	0.530	0.716
Wean	179.2	178.2	180.6	181.8	2.49	0.335	0.738
BW loss, kg							
d 0 to 10	-2.03	-2.23	-1.43	-1.31	0.958	0.439	0.860
d 10 to wean	-3.08	-2.83	-2.28	-2.08	0.878	0.318	0.971
d 0 to wean	-5.12	-5.06	-3.76	-3.38	1.377	0.235	0.899
ADFI, kg							
d 0 to 10	4.66	4.44	4.50	4.64	0.127	0.952	0.130
d 10 to wean	6.36	6.27	6.21	6.31	0.173	0.743	0.573
d 0 to wean	4.99	4.74	4.90	4.97	0.136	0.821	0.226
Total Lys intake ² , g/d	50.4	53.5	61.0	71.2	1.61	0.001	0.016
SID Lys intake ³ , g/d	39.9	45.0	53.9	62.1	1.35	0.001	0.243
BF loss, mm							
d 0 to 10	-0.99	-1.62	-0.95	-1.09	0.249	0.549	0.306
d 10 to wean	-1.48	-0.94	-1.23	-0.60	0.265	0.087	0.874
d 0 to wean	-2.51	-2.53	-2.18	-1.65	0.329	0.046	0.410
Lactation length, d	18.7	18.8	18.6	18.4	0.34	0.946	0.534
Wean-to-estrus interval, d	5.00	4.91	4.97	4.61	0.45	0.691	0.800
Females bred by 7 d after weaning, %	89.9	89.9	94.2	100.0	6.05	0.975	0.977
d 30 conception rate ⁴ , %	87.5	96.0	96.3	78.8	8.0	0.537	0.051
Farrowing rate ⁵ , %	79.2	88.0	96.0	74.8	8.03	0.789	0.290

¹A total of 111 primiparous sows (DNA 241, DNA Genetics) across 4 farrowing groups were used in a 21-d trial with 27 to 29 sows per dietary treatment.

²Calculated using analyzed Lys values and ADFI.

³Calculated using formulated SID Lys values and ADFI.

⁴Number of sows confirmed pregnant on d 30 post mating divided by number of sows bred.

⁵Number of sows farrowed divided by number of sows bred by d 21 after weaning.

Table 3-6 Effects of increasing standardized ileal digestible (SID) lysine in lactation diets on litter and subsequent performance, Exp. 1¹

Item	SID Lysine, %				SEM	Probability, <i>P</i> <	
	0.80	0.95	1.10	1.25		Linear	Quadratic
Litter size, n							
d 2	13.1	13.3	13.0	12.3	0.70	0.983	0.953
d 10	13.1	13.3	13.0	12.3	0.70	0.983	0.953
Wean	12.9	13.1	12.9	13.2	0.70	0.916	0.988
Litter weight, kg							
d 2	19.2	19.4	18.5	18.7	0.49	0.263	0.965
d 10	42.0	41.6	41.4	41.5	0.53	0.451	0.528
Wean	69.5	69.0	67.4	67.7	1.31	0.120	0.728
Litter gain, kg							
d 2 to 10	22.8	22.5	21.9	22.1	0.57	0.267	0.617
d 10 to wean	27.6	27.7	25.7	26.1	1.22	0.194	0.899
d 2 to wean	50.2	49.9	48.0	48.4	1.36	0.209	0.766
Litter ADG d 2 to wean, g	2,984	2,959	2,896	2,938	57.3	0.374	0.545
Subsequent performance ²							
Total piglets born per sow farrowed, n	14.3	16.4	15.2	15.7	0.95	0.603	0.394
Born alive, %	94.2	89.8	91.0	93.7	2.05	0.955	0.054
Stillborn, %	5.0	7.2	7.0	5.0	1.73	0.998	0.193
Mummy, %	0.6	3.0	1.5	1.0	1.03	0.960	0.090

¹A total of 111 primiparous sows (DNA 241, DNA Genetics) across 4 farrowing groups were used in a 21-d trial with 27 to 29 sows per dietary treatment.

²Number of sows included for subsequent performance were 19, 22, 26, and 20 for dietary treatments of 0.80, 0.95, 1.10 and 1.25% SID Lys, respectively.

Table 3-7 Effects of increasing lysine on sow performance in lactation of high-performing gilts and sows under commercial conditions, Exp. 2¹

	Standardized ileal digestible Lys, %				SEM	Probability, <i>P</i> <	
	0.75	0.90	1.05	1.20		Linear	Quadratic
Count, n	187	185	194	144	---	---	---
Parity	3.1	3.2	3.2	3.2	0.15	0.576	0.928
Sow BW, kg							
d 112 ²	209	209	209	208	1.8	0.478	0.932
Post-farrow ^{2,3}	195	194	194	193	1.8	0.487	0.958
Wean ²	173	176	180	177	2.0	0.017	0.046
Sow BW change, kg							
Post-farrow ³ to wean ²	-21.3	-18.2	-14.6	-16.9	1.45	0.001	0.018
d 112 to wean ²	-35.7	-31.9	-28.5	-31.6	1.50	0.003	0.004
Sow back fat ⁴ , mm							
d 112 ²	20.0	21.2	20.3	20.1	0.65	0.676	0.184
Wean ²	18.6	18.4	17.6	18.0	0.45	0.121	0.395
Change (d 112 to wean) ²	-1.4	-2.6	-2.8	-2.6	0.44	0.028	0.061
Loin eye depth, mm							
d 112 ²	52.9	52.4	52.3	52.6	0.77	0.722	0.575
Wean ²	50.2	51.2	52.0	52.6	0.64	0.002	0.784
Change (d 112 to wean) ²	-1.9	-1.0	-0.1	0.5	0.61	0.002	0.784
Lactation length, d	21.3	21.4	21.4	21.4	0.11	0.485	0.435
Females bred by d 7 after weaning ² , %	88.9	92.6	94.8	92.4	2.60	0.227	0.199
d 30 conception rate ⁵ , %	94.7	89.7	95.8	90.8	2.86	0.928	0.700
Farrowing rate ⁶ , %	92.3	85.6	93.8	88.6	3.39	0.957	0.951
ADFI from feed delivery records ⁷ , kg	6.45	6.36	6.68	5.90	---	---	---
SID Lys intake ⁸ , g/d	48.4	57.2	70.2	70.6	---	---	---

¹A total of 710 sows (DNA 241) and litters were used in a lactation study from d 112 of gestation until weaning.

²Significant differences of treatment within parity category observed.

³Post-farrow weight was calculated using d 112 BW and subtracting weight of conceptus (calculated using modified equation by Thomas et al., 2016).

⁴A subsample of sows (n = 369) were ultra-sounded on d 112 for backfat and loin eye depth and subsequently used in the backfat and loin eye depth change calculation. All 710 sows were measured at weaning for backfat and loin eye depth.

⁵Number of sows confirmed pregnant on d 30 post mating divided by number of sows bred.

⁶Number of sows farrowed divided by number of sows bred by d 21 after weaning.

⁷Calculated using total feed deliveries by treatment and dividing by total number of sows on feedline.

⁸Calculated using ADFI multiplied by SID Lys in the experimental diet.

Table 3-8 Effects of increasing lysine in lactation on litter performance of high-performing gilts and sows under commercial conditions, Exp. 2¹

	Standardized ileal digestible Lys, %				SEM	Probability, <i>P</i> <	
	0.75	0.90	1.05	1.20		Linear	Quadratic
Total born	15.9	15.4	15.4	16.4	0.38	0.497	0.255
Litter size ² , n							
d 2	13.6	13.7	13.7	13.7	0.07	0.950	0.965
d 17	12.6	12.7	12.7	12.7	0.11	0.896	0.945
Litter weight, kg							
d 2	21.5	21.8	22.2	21.2	0.34	0.835	0.016
d 17	61.3	61.5	64.1	60.2	0.64	0.807	0.001
Litter gain d 2 to 17, kg	39.7	39.8	42.5	38.6	0.64	0.807	0.001
Litter ADG d 2 to 17, g	2,695	2,704	2,887	2,619	43.4	0.807	0.001
Subsequent performance ³							
Total born per sow farrowed, n	15.9	16.0	16.3	15.1	0.41	0.482	0.310
Born alive, %	92.0	93.0	92.0	92.4	0.78	0.863	0.666
Stillborns, %	4.3	3.3	4.2	5.1	0.63	0.150	0.065
Mummies, %	3.5	3.6	3.7	2.4	0.51	0.158	0.110

¹A total of 710 sows (DNA 241) and litters were used in a lactation study from d 112 of gestation until weaning.

²Litters were cross-fostered to equalize litter size up to 48-h post-farrowing.

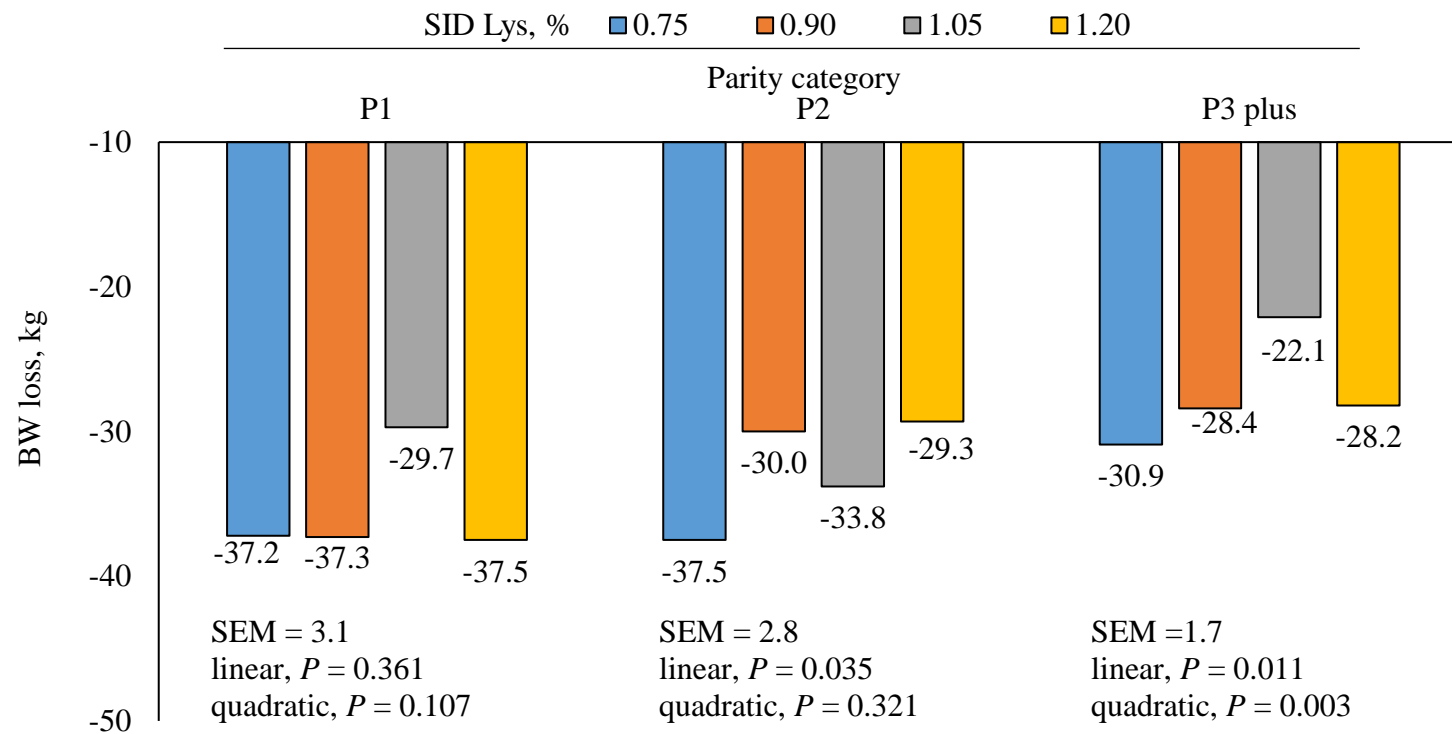
³Number of sows included in subsequent performance are 161, 149, 140, 108 for dietary treatments of 0.75, 0.90, 1.05 and 1.20% SID Lys, respectively.

Table 3-9 Least square mean estimates of the effects of SID lysine level during lactation of high-performing parity 1, parity 2 and parity 3+ sows under commercial conditions, Exp. 2^{1,2}

Item	Parity 1						Parity 2						Parity 3+						
	0.75	0.90	1.05	1.20	Linear	Quadratic	0.75	0.90	1.05	1.20	Linear	Quadratic	0.75	0.90	1.05	1.20	Linear	Quadratic	
SID Lys, %																			
Count, n	38	39	39	27			44	43	46	33			105	103	109	84			
Sow BW, kg																			
d 112	197	196	194	198	0.911	0.374	205	201	203	200	0.288	0.938	226	228	228	226	0.772	0.133	
SEM	3.4	3.4	3.4	3.8			3.2	3.2	3.2	3.4			2.0	2.0	2.0	2.1			
Post-farrow ³	183	183	181	185	0.895	0.348	190	186	188	185	0.287	0.937	211	213	213	211	0.766	0.133	
SEM	3.4	3.4	3.4	3.8			3.2	3.2	3.1	3.4			2.0	2.0	2.0	2.1			
Wean	159	159	164	162	0.209	0.756	165	170	169	170	0.365	0.457	196	200	206	199	0.014	0.001	
SEM	3.6	3.6	3.6	4.1			3.3	3.3	3.3	3.7			2.1	2.1	2.1	2.3			
Sow BW change, kg																			
Post-farrow ³ to wean	-24.5	-25.8	-18.6	-24.4	0.223	0.237	-23.8	-15.6	-18.8	-14.1	0.022	0.413	-14.8	-13.2	-6.3	-12.3	0.009	0.007	
SEM	2.54	2.53	2.53	2.96			2.36	2.38	2.32	2.70			1.52	1.54	1.49	1.69			
d 112 to wean	-37.2	-37.3	-29.7	-37.5	0.361	0.107	-37.5	-30.0	-33.8	-29.3	0.035	0.321	-30.9	-28.4	-22.1	-28.2	0.011	0.003	
SEM	2.6	2.6	2.6	3.1			2.4	2.5	2.4	2.8			1.6	1.6	1.5	1.7			
Backfat d 112 ⁴ , mm	21.9	23.3	22.2	24.3	0.270	0.756	20.3	21.4	20.2	18.5	0.150	0.162	17.8	18.9	18.5	17.4	0.597	0.142	
SEM	1.16	1.12	1.15	1.27			1.06	1.10	1.11	1.23			0.75	0.79	0.76	0.84			
Backfat wean ⁴ , mm	20.3	19.9	18.9	20.5	0.840	0.189	18.4	17.9	16.9	17.5	0.238	0.442	17.1	17.3	17.0	16.0	0.087	0.208	
SEM	0.79	0.78	0.77	0.93			0.73	0.74	0.72	0.84			0.47	0.48	0.47	0.53			
Backfat change d 112 to wean ⁴ , mm	-1.8	-3.6	-3.8	-4.3	0.029	0.363	-1.8	-3.0	-3.2	-1.7	0.978	0.057	-0.5	-1.0	-1.5	-1.7	0.080	0.817	
SEM	0.77	0.74	0.77	0.85			0.71	0.73	0.74	0.82			0.50	0.53	0.51	0.56			
Loin eye depth d 112, mm	55.7	53.8	56.8	58.5	0.034	0.152	52.7	51.9	49.7	48.2	0.004	0.782	50.3	51.6	50.5	51.0	0.894	0.667	
SEM	1.36	1.31	1.36	1.50			1.25	1.30	1.21	1.46			0.88	0.94	0.89	0.99			
Loin eye depth wean, mm	48.6	48.1	51.4	51.7	0.009	0.718	49.9	52.4	51.1	51.7	0.471	0.394	52.1	53.0	53.5	54.5	0.029	0.953	
SEM	1.13	1.08	1.14	1.27			1.03	1.07	1.10	1.21			0.73	0.78	0.74	0.83			
Loin eye depth change d112 to wean, mm	-3.5	-4.0	-0.7	-0.4	0.009	0.718	-2.3	0.2	-1.1	-0.4	0.471	0.394	0.1	0.9	1.4	2.4	0.029	0.953	
SEM	1.13	1.08	1.14	1.27			1.03	1.07	1.10	1.22			0.73	0.78	0.74	0.82			
Litter weight d 2, kg	20.8	21.0	21.7	19.5	0.329	0.041	22.2	23.1	23.8	23.2	0.145	0.188	21.4	21.4	21.2	20.8	0.252	0.572	
SEM	0.59	0.58	0.58	0.70			0.55	0.56	0.54	0.63			0.36	0.36	0.35	0.40			
Piglet fallout rate, %	1.4	2.6	0.9	3.4	0.568	0.512	3.9	2.2	5.4	2.0	0.683	0.532	2.2	2.8	2.2	2.5	0.933	0.746	
SEM	0.67	0.98	0.54	1.37			1.07	0.82	1.23	0.88			0.49	0.55	0.47	0.55			
Females bred by d 7 after weaning, %	76.5	84.1	92.3	92.7	0.047	0.831	88.8	95.4	95.6	90.8	0.799	0.183	95.2	95.7	95.9	93.7	0.743	0.559	
SEM	7.55	6.64	4.43	5.30			5.01	3.29	3.16	5.27			2.18	2.18	2.04	2.85			

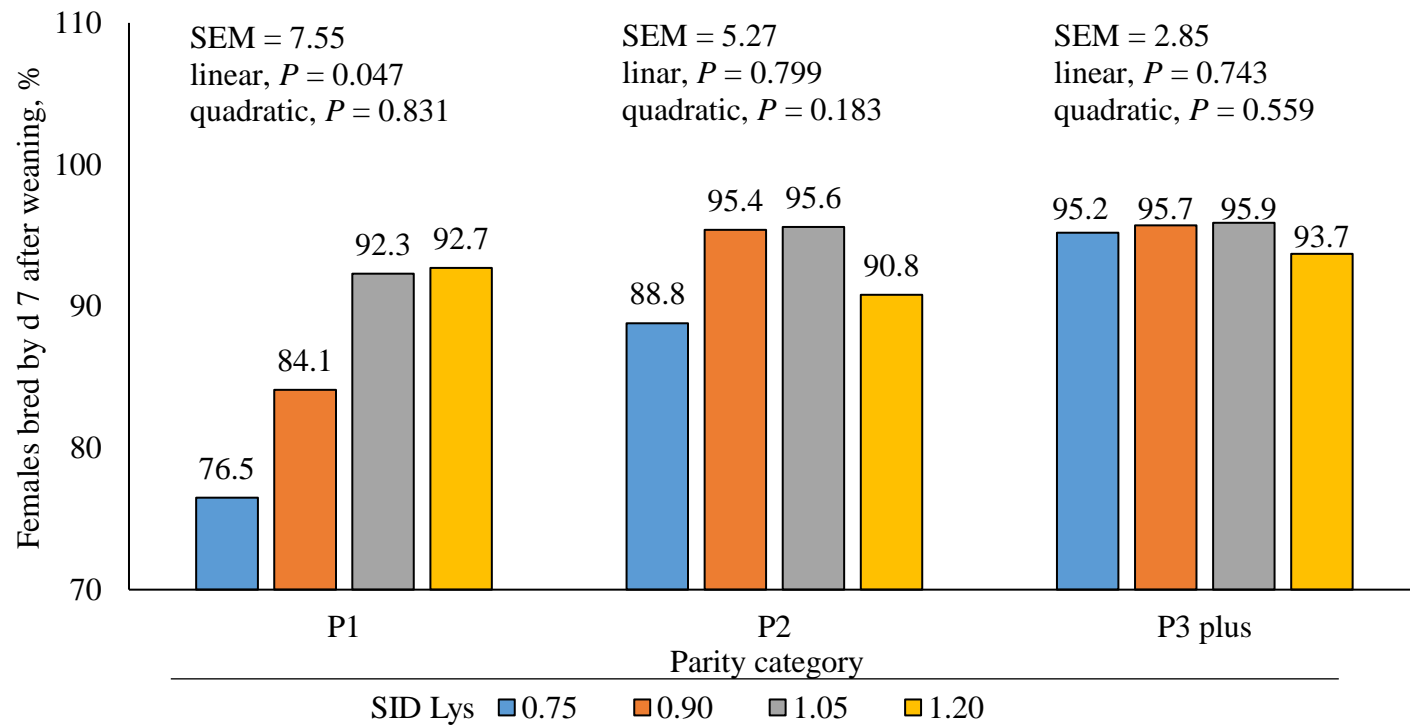
¹A total of 710 sows (DNA 241) and litters were used in a lactation study from d 112 of gestation until weaning.²Only variables that have a significant difference observed within treatment across parity category are shown.³Post-farrow weight was calculated using d112 BW and subtracting weight of conceptus (calculated using modified equation by Thomas et al., 2016).

Figure 3-1 Estimated mean sow BW loss from d 112 of gestation until weaning within parity category for sows fed increasing SID Lys in lactation, Exp. 2¹



¹A total of 710 sows (DNA 241) and litters were used in a lactation study from d 112 of gestation until weaning. Sows were fed experimental diets from d 114 of gestation until weaning (approximately 21 d).

Figure 3-2 Estimated percentage females bred by d 7 after weaning within parity category for sows fed increasing SID Lys in lactation, Exp. 2¹



¹A total of 710 sows (DNA 241) and litters were used in a lactation study from d 112 of gestation until weaning. Sows were fed experimental diets from d 114 of gestation until weaning (approximately 21d).