

Dose-responses to lysine, valine, and isoleucine and the effects of
monosodium glutamate on nursery pigs

by

Anne Bonner Clark

B.S., Kansas State University, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Co-Major Professor
Dr. Joel DeRouchey

Approved by:

Co-Major Professor
Dr. Mike Tokach

Copyright

© Anne Bonner Clark 2016.

Abstract

Six experiments using a total of 2,974 nursery pigs were used to determine the effects of monosodium glutamate (MSG) and amino acids (AA) on nursery pig growth performance. Experiments 1 and 2 evaluated increasing dietary MSG for nursery pigs. Increasing dietary MSG up to 2% without balancing for sodium and chloride content decreased nursery pig performance, and feeding sodium levels equivalent to 1% MSG also decreased performance. When sodium and chloride were balanced, there were marginal effects of increasing dietary MSG on pig performance. Experiment 3 was conducted to determine the standardized ileal digestible (SID) lysine (Lys) requirement for pigs weighing 7- to 11- kg. The SID Lys requirement was estimated to be 1.45% and greater than 1.60% depending on the statistical model applied for both ADG and G:F. This experiment served to validate the SID Lys requirement for use in formulating diets for the subsequent experiments. Experiment 4 evaluated increasing SID valine (Val) to Lys ratio for nursery pigs weighing 7- to 10- kg. A SID Val:Lys ratio of 62.9% optimized ADG. Maximum feed efficiency (G:F) was captured using 71.7% SID Val:Lys ratio, however, 99% of maximum was achieved with SID Val at 64.4% of Lys. For ADFI, maximum performance was at 74% SID Val:Lys ratio, with 99% of maximum intake achieved at 68%. Experiments 5 and 6 investigated increasing SID isoleucine (Ile) to Lys ratio for 6- to 11- kg pigs. When ADG and ADFI were modeled, broken-line models reported maxima of 52.0% Ile:Lys ratio while quadratic models were as high as 64% of Lys.

Table of Contents

List of Figures	vi
List of Tables	vii
Acknowledgements	viii
Dedication	ix
Chapter 1 - Effects of monosodium glutamate on nursery pig growth performance.....	1
Abstract	1
Introduction.....	2
Materials and Methods.....	3
Experiment 1	3
Experiment 2	4
Chemical Analysis	5
Statistical Analysis	5
Results.....	5
Chemical Analysis	5
Growth Performance	6
Experiment 1	6
Experiment 2	6
Discussion.....	7
Literature Cited	12
Chapter 2 - Modeling the effects of standardized ileal digestible valine to lysine ratio on growth performance of nursery pigs	28
Abstract	28
Introduction.....	29
Materials and Methods.....	30
General	30
Experiment 1	32
Experiment 2	32
Statistical Analysis	32
Results and Discussion	34

Literature Cited	41
Chapter 3 - Modeling the effects of standardized ileal digestible isoleucine to lysine ratio on growth performance of nursery pigs	58
Abstract	58
Introduction.....	59
Materials and Methods.....	60
General	60
Experiment 1	61
Experiment 2	62
Statistical Analysis	62
Results.....	64
Experiment 1	64
Experiment 2	66
Discussion.....	67
Literature Cited	71

List of Figures

Figure 2-1. Estimated standardized ileal digestible (SID) Lys requirement to maximize ADG for nursery pigs ¹	53
Figure 2-2. Estimated standardized ileal digestible (SID) Lys requirement to maximize feed efficiency (G:F) for nursery pigs ¹	54
Figure 2-3. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize ADG for nursery pigs ¹	55
Figure 2-4. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize ADFI for nursery pigs ¹	56
Figure 2-5. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize feed efficiency (G:F) for nursery pigs ¹	57
Figure 3-1. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 1 ¹	84
Figure 3-2. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 1 ¹	85
Figure 3-3. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 2 ¹	86
Figure 3-4. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 2 ¹	87

List of Tables

Table 1-1. Phase 1 diet composition (as-fed basis), Exp. 1 ¹	14
Table 1-2. Phase 2 and 3 diet composition (as-fed basis), Exp. 1 ¹	16
Table 1-3. Phase 1 diet composition (as-fed basis), Exp. 2 ¹	18
Table 1-4. Phase 2 diet composition (as-fed basis), Exp. 2 ¹	20
Table 1-5. Phase 3 diet composition (as-fed basis), Exp. 2 ¹	22
Table 1-6. Chemical analysis of diets (as-fed basis), Exp. 1.....	24
Table 1-7. Chemical analysis of diets (as-fed basis), Exp. 2.....	25
Table 1-8. Effects of monosodium glutamate on nursery pig performance, Exp. 1 ¹	26
Table 1-9. Effects of monosodium glutamate on nursery pig performance, Exp. 2 ^{1,2}	27
Table 2-1. Chemical analysis of ingredients for Exp. 2 ^{1,2}	44
Table 2-2. Diet composition (as-fed basis), Exp. 1 ¹	45
Table 2-3. Diet composition (as-fed basis), Exp. 2 ¹	47
Table 2-4. Chemical analysis of diets (as-fed basis), Exp. 1 ¹	49
Table 2-5. Chemical analysis of diets (as-fed basis), Exp. 2 ¹	50
Table 2-6. Effects of standardized ileal digestible (SID) Lys on nursery pig growth performance ^{1,2}	51
Table 2-7. Effects of standardized ileal digestible (SID) Val:Lys ratio on nursery pig growth performance ^{1,2}	52
Table 3-1. Chemical analysis of ingredients.....	75
Table 3-2. Diet composition (Exp. 1, as-fed basis) ¹	76
Table 3-3. Diet composition (Exp. 2, as-fed basis) ¹	78
Table 3-4. Chemical analysis of diets (Exp. 1, as-fed basis) ¹	80
Table 3-5. Chemical analysis of diets (Exp. 2, as-fed basis) ¹	81
Table 3-6. Effects of increasing standardized ileal digestible (SID) Ile:Lys ratio on nursery pig growth performance, Exp. 1 ¹	82
Table 3-7. Effects of increasing standardized ileal digestible (SID) Ile:Lys ratio on nursery pig growth performance, Exp. 2 ¹	83

Acknowledgements

This thesis would not have been possible without the help of several individuals. I would like to thank my major professors, Dr. Joel DeRouchey and Dr. Mike Tokach, for their guidance, encouragement, and support throughout my program. Furthermore, I am extremely grateful to the members of my committee, Drs. Dritz, Goodband, and Woodworth, for their instruction and dedication of time. I strongly believe in the education and training I have received at K-State and feel fortunate to be a part of the family atmosphere.

I am tremendously thankful for the graduate students of the swine nutrition team who came before me and established a tradition of success and camaraderie and for the current members who make this journey not only possible, but absolutely enjoyable.

Lastly, to Mom, Dad, and Bracey, I would not be who I am without your love and support. I am forever grateful.

Dedication

This thesis is dedicated to my grandparents, Donald and Hazel Clark, and the memories of Robert and Elizabeth Bonner.

Chapter 1 - Effects of monosodium glutamate on nursery pig growth performance

Abstract

Two experiments were conducted to evaluate the effects of monosodium glutamate (MSG) on nursery pig growth performance. In Exp. 1, 1,134 nursery pigs (PIC 280 × 1050, initially 5.1 kg BW) were allotted to 1 of 6 treatments and fed for 48-d. There were 27 pigs/pen and 7 pens/treatment. Dietary treatments contained 0, 0.5, 1.0, 1.5, and 2.0% MSG, or a high salt diet, formulated to equal Na content as the 1.0% MSG treatment. Experimental diets were fed in 3 phases from d 0 to 12, d 12 to 26, and d 26 to 48. During phase 1, no evidence for differences was detected among MSG treatments, but pigs fed the high salt diet (0.78% added salt) tended ($P = 0.069$) to have poorer G:F than pigs fed the 1% MSG treatment. In phase 2, increasing MSG decreased (linear, $P < 0.045$) ADG, ADFI, and G:F while pigs fed the high salt diet (0.84% added salt) had decreased ($P < 0.001$) ADG and G:F compared with pigs fed the 1% MSG diet. In phase 3, no evidence for difference was detected among the MSG treatments; however, pigs fed the high salt diet (0.84% added salt) had decreased ($P < 0.028$) ADG and ADFI compared with those fed the 1% MSG diet. Pig BW was reduced (linear, $P < 0.016$) on d 26 and 48 for pigs fed increasing MSG and pigs fed the high salt treatment had decreased ($P < 0.001$) BW compared to pigs fed 1% MSG. For the overall nursery period, increasing MSG decreased (linear, $P = 0.033$) ADG and tended (linear, $P = 0.095$) to decrease ADFI. Furthermore, pigs fed the high salt diet had decreased ($P < 0.009$) ADG, ADFI, and G:F compared to their 1% MSG counterparts. In Exp. 2, 700 nursery pigs (PIC C-29 × 1050, initially 6.2 kg BW) were allotted to 1 of 5 treatments fed for 42-d. There were 10 pigs/pen and 14 pens/treatment. Dietary treatments contained 0, 0.5, 1.0, 1.5, and 2.0% MSG and were balanced for Na and Cl content by using

sodium bicarbonate and potassium chloride. Experimental diets were fed in 3 phases from d 0 to 14, d 14 to 28, and d 28 to 42. For ADG and ADFI, there was no evidence for differences within any phase or overall. Increasing MSG did not affect G:F during phase 1; however, it tended (quadratic, $P = 0.059$) to improve G:F in phase 2, but resulted in poorer G:F (linear, $P = 0.003$) for phase 3. Thus, for the overall nursery period, G:F tended (quadratic, $P = 0.080$) to be poorer with increasing MSG. In conclusion, MSG did not improve nursery pig performance and MSG may reduce intake and gain when dietary Na is not balanced.

Key words: chloride, growth, monosodium glutamate, nursery pig, sodium

Introduction

Glutamate serves many roles in cellular processes and is particularly important for intestinal tract function and gut development. While considered a non-essential amino acid, glutamate significantly contributes to the energy supply for intestinal cells (Watford, 2008). The suckling pig receives sufficient glutamate from the sow's milk (Wu and Knabe, 1994), however, due to decreased feed intake typically seen immediately after weaning, nursery pigs are often limited in glutamate intake. This may exacerbate common post-weaning issues, such as impaired growth performance and diarrhea due to damaged intestinal villi. While there is substantial understanding of glutamate, research involving the administration of glutamate via monosodium glutamate (MSG) is limited. Rezaei et al. (2013) observed that supplementing up to 4% dietary MSG improved nursery pig growth performance.

Besides glutamate, MSG contains 19.2% Na. Thus, inclusion of MSG in the diet can dramatically increase the Na content of the diet. Whether the high level of Na in MSG may be a concern for pig performance is unknown. The NRC (2012) lists the Na requirement at 0.40, 0.35, and 0.28 for 5- to 7- kg, 7- to 11- kg, and 11- to 25- kg pigs, respectively. Nutritionists must

therefore determine whether to alter the inclusion of other dietary Na sources, such as salt, when adding MSG to the diet. Therefore, the objectives of these experiments were to determine the effects of increasing monosodium glutamate addition to diets not balanced or balanced for dietary Na and Cl on nursery pig performance.

Materials and Methods

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

Experiment 1

A total of 1,134 nursery pigs (PIC 280 × 1050, initially 5.1 kg BW) were used in a 48-d growth trial with 27 pigs per pen and 7 replications per treatment. Pigs were weaned at approximately 17 d of age and were randomly allotted to pens upon weaning. Pens were then blocked by BW and allotted to one of 6 dietary treatments. Pigs were fed in 3 phases from d 0 to 12, 12 to 26, and 26 to 48 with treatments fed for all 3 phases. Dietary treatments contained 0, 0.5, 1.0, 1.5, and 2.0% MSG, or a high salt diet. The 0 and 2.0% MSG diets were blended by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) to create the 3 intermediate MSG treatments. The high salt treatment was formulated to contain the equivalent amount of Na as the 1% MSG treatment: 0.58, 0.50, and 0.42% Na in phases 1, 2, and 3, respectively (Tables 1-1 and 1-2) which resulted in the addition of 0.78% salt in phase 1 and 0.84% in both phases 2 and 3. Diets were formulated at 1.40, 1.35, and 1.25% standardized ileal digestible (SID) Lys in phases 1, 2, and 3, respectively, with other essential AA formulated above the pig's requirement estimate (NRC, 2012). Phase 1 was fed in pelleted form, and phases 2 and 3 were fed in meal

form. Pens were weighed and feed disappearance was measured on d 0, 12, 26, and 48 to determine ADG, ADFI, and G:F.

The trial was conducted at a commercial nursery research facility in southwest Minnesota. The barn was mechanically ventilated and had completely slatted flooring and deep pits for manure storage. Each pen was equipped with a 6-hole, stainless-steel, dry self-feeder and a pan waterer allowing ad libitum access to feed and water. Diets were manufactured at two commercial feed mills (Hubbard, Mankato, Minnesota for phase 1; and New Horizon Farms, Pipestone, MN for phases 2 and 3).

Experiment 2

A total of 700 nursery pigs (PIC C-29 × 1050, initially 6.2 kg BW) were used in a 42-d growth study trial with 10 pigs per pen and 14 replications per treatment. Pigs were weaned at approximately 21-d of age and allotted to pens upon weaning. Pens were blocked by BW and allotted to one of 5 dietary treatments: 0, 0.5, 1.0, 1.5, and 2.0% MSG. Pigs were fed in three phases from d 0 to 14, 14 to 28, and 28 to 42. Pens were weighed and feed disappearance was measured on d 0, 14, 28, and 42 to determine ADG, ADFI, and G:F. Phase 1 diets were pelleted, while phases 2 and 3 were fed in meal form. Diets (Tables 1-3, 1-4, and 1-5) were formulated to balance Na and Cl content with increasing MSG utilizing salt, sodium bicarbonate, and potassium chloride. Diets were formulated at 1.40, 1.35, and 1.25% SID Lys in phases 1, 2, and 3, respectively, with other essential AA formulated above the pig's requirement estimates (NRC, 2012). Diets were manufactured at a commercial feed mill (Kalmbach Feeds, Inc., Upper Sandusky, Ohio).

The trial was conducted at a commercial research facility that is owned and operated by Kalmbach Feeds, Inc. (Sycamore, OH). Each pen was equipped with a 6-hole, stainless-steel, dry self-feeder and a pan waterer allowing ad libitum access to feed and water.

Chemical Analysis

Samples of each diet were collected for each phase, blended, and sub-sampled prior to analysis (Ward Laboratory, Kearney, NE) for DM (method 935.29; AOAC Int., 2012), CP (method 990.03; AOAC Int., 2012), ether extract [method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY)], crude fiber [method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY)], Ca and P [method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA)], ADF and NDF (Van Soest, 1963), Na (method 990.08, AOAC Int., 2012), and Cl (method 969.10, AOAC Int., 2012).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen considered the experimental unit. Linear and quadratic contrasts were applied for the MSG treatments. Both experiments were analyzed as randomized complete block designs. In Exp.1, a single degree of freedom contrast was used to compare the high salt diet to the 1% MSG diet. In Exp. 2, initial BW was used as a covariate. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results

Chemical Analysis

In Exp. 1, diet analysis generally matched formulated nutrient levels, with some variability exhibited in Na concentration (Table 1-6). Particularly, in the phase 1 diets, where the

Na content was lower than formulated values, and the phase 3 diets, where the high MSG treatments analyzed lower in Na than formulated. Nevertheless, Na content increased as MSG content increased in the diets as expected. In Exp. 2, dietary analysis was also reasonably consistent with formulated levels of nutrients (Table 1-7). In the phase 1 diets, Na levels although balanced in the diet were slightly variable around the expected value of 0.43%. Na levels were relatively constant in the phase 2 diets and lower than formulated values for the high MSG treatments in phase 3, with Cl analyzing consistently.

Growth Performance

Experiment 1. During phase 1 (d 0 to 12), adding MSG to the diet did not affect pig performance; however, pigs fed the high salt diet tended ($P = 0.069$) to have poorer G:F than pigs fed 1.0% MSG (Table 1-8). In phase 2 (d 12 to 26), increasing MSG decreased (linear, $P < 0.045$) ADG, ADFI, and G:F. In addition, pigs fed the high salt diet had poorer ($P < 0.001$) ADG and G:F than pigs fed 1.0% MSG. In phase 3 (d 26 to 48), no evidence for differences was detected among the MSG treatments. Pigs fed the high salt diet had decreased ($P < 0.028$) ADG and ADFI compared with those fed 1% MSG. No evidence for differences was observed in BW until d 26 and 48, where increasing MSG decreased (linear, $P < 0.016$) BW and pigs fed the high salt diets had decreased ($P < 0.001$) BW compared to pigs fed 1.0% MSG. For the overall nursery period (d 0 to 48), increasing MSG decreased (linear, $P = 0.033$) ADG and tended to decrease (linear, $P = 0.095$) ADFI. Furthermore, pigs fed the high salt diet had decreased ($P < 0.009$) ADG and ADFI and poorer ($P < 0.001$) G:F compared to their 1.0% MSG counterparts.

Experiment 2. During phase 1 (d 0 to 14), increasing MSG did not influence ADG, ADFI, or G:F (Table 1-9). In phase 2 (d 14 to 28), there was no evidence for differences between dietary treatments for ADG or ADFI; however, G:F tended (quadratic, $P = 0.059$) to improve

with increasing MSG. For phase 3 (d 28 to 42), once again there was no evidence of a dietary effect on ADG or ADFI, but G:F decreased (linear, $P = 0.003$) with increasing MSG. For the overall nursery period (d 0 to 42), increasing MSG had no effect on ADG or ADFI and tended (quadratic, $P = 0.080$) to decrease G:F. No evidence for difference was observed in BW between any of the dietary treatments.

Discussion

Glutamate, a nonessential AA, serves as primary fuel for oxidative processes in the gastrointestinal tract and functions as a neurotransmitter (Burrin and Stoll, 2009). Thus, research with dietary glutamate has been conducted to determine its role in the gastrointestinal tract. In addition to being an intestinal energy source, glutamate serves as a precursor within the small intestine for several other nutrients, such as glutathione, arginine, and proline (Reeds et al. 2000). These functions become particularly important in developing animals where the intestinal tract demonstrates increased sloughing and regeneration of epithelial cells, often exacerbated at weaning.

Wu et al. (2012) supplemented nursery pig diets with 1% dietary glutamate, resulting in increased jejunal villus height and mucosal thickness. Pigs fed glutamate also demonstrated increased proliferating cell nuclear antigen mRNA and β -catenin mRNA present in the jejunum. Similarly, Liu et al. (2002) supplemented weaned pigs with 1% L-glutamate and found a reduction in jejunal atrophy and an increase in capacity to absorb D-xylose and concentration of RNA in skeletal muscle tissue. Furthermore, Lin et al. (2014) found that weanling pigs who received 2% L-glutamate exhibited increased levels of glutamate oxaloacetate transaminase in jejunal mucosa, mRNA expression of jejunal mucosa glutamine synthetase and its receptors, duodenal and jejunal villus height, and plasma concentrations of several AA. This literature and

others influenced Wu (2014) to describe the concept of establishing requirements for AA such as glutamate that are traditionally considered nonessential AA. Although the body can synthesize glutamate, it may not be in sufficient quantities for metabolic processes especially in nursery pigs. These findings indicate that glutamate plays a significant role in gut health and supplementation may lead to improvements in intestinal structure and function. Considering the associated physiological stresses associated with weaning, it is reasonable that the addition of glutamate to swine diets would prove beneficial, particularly during the nursery period.

Along with L-glutamate, MSG is another potential dietary source of glutamate. However, there is currently limited literature regarding its use in swine diets. Zhang et al. (2013) determined that oral administration of MSG at up to 1 g/kg BW for suckling piglets increased the expression of glutamate receptors and AA transporters in the stomach and jejunum, allowing for more utilization of glutamate and a reduction of subsequent nutrient loss to the large intestine. Likewise, Rezaei et al. (2013) conducted an experiment in which supplementing up to 4% MSG resulted in improved ADG and feed efficiency during the post-weaning period; however, ADFI was reduced at the 4% addition. They found that with increasing dietary MSG, there was a reduction in the prevalence of diarrhea during the first week in the nursery, as well as increased jejunal villus height, DNA content, and antioxidative capacity 7 d post-weaning. Additionally, increasing MSG in the diet increased plasma concentrations of glutamate, glutamine, and other AA. However, they did not find differences in other hematological response criteria, clinical chemistry tests, and gross and microscopic structures, including sodium concentration within plasma even though the diets in this experiment contained 0.25% salt in addition to the Na provided by MSG. These benefits in the intestinal morphology agree with the previously

discussed literature regarding L-glutamate supplementation, and suggest that the benefits of L-glutamate can potentially be observed when administered in the form of MSG.

Our results do not agree with those of Rezaei et al. (2013), who found improved performance in nursery pigs with up to 4% supplementation of MSG. Their basal diets contained 0.25% NaCl and the addition of MSG at 0, 1, 2, and 4% and were not balanced for Na, similar to Exp. 1. Considering how the performance of the high salt treatment in Exp. 1 was significantly poorer than the 1% MSG treatment containing equal Na content, the negative effects of 4% MSG in our experiment may have been due to increased Na. This presents the possibility that MSG could be beneficial as the addition of salt decreased performance and could explain the lack of response seen in Exp. 2 where performance was mostly unchanged with increasing MSG when Na and Cl were held constant. Another difference noted is that the Rezaei et al. (2013) experiment used simpler diets containing primarily soybean meal as the protein source, whereas our diets contained dried whey, fishmeal, and enzymatically treated soybean meal. This may have resulted in less gastrointestinal challenge to our pigs as compared to soybean meal alone, which minimized the potential effects of MSG.

The NRC (2012) cites that the Na requirement is 0.40%, 0.35%, and 0.28% for 5- to 7-kg, 7- to 11-kg, and 11- to 25-kg pigs, respectively. Pigs demonstrate ability to tolerate high levels of dietary Na, as long as adequate drinking water is provided (NRC, 2005). Furthermore, the Cl requirement estimates for these weight ranges are 0.50%, 0.45%, and 0.32%, respectively. In Exp. 1, Na content ranged from 0.39 to 0.78% in phase 1, 0.31 to 0.69% in phase 2, and 0.23 to 0.61% in phase 3. While Exp. 1 treatments with 0% MSG were marginally under the NRC Na requirement, it did not negatively impact performance, but rather performance became poorer with increasing Na. The Cl content was well above NRC (2012) recommendations at 0.78, 0.62,

and 0.54% in the MSG diets and 1.06, 0.91, and 0.83% in the high salt diet in phase 1, 2, and 3, respectively. In Exp. 2, Na and Cl concentrations were balanced to the NRC (2012) requirements as much as possible while still increasing MSG from 0 to 2%. In phase 1, Na and Cl were 0.43 and 0.50%, respectively, in all treatments. However, in phase 2, Na content was 0.35% in the first four levels of MSG and 0.43% in the 2% MSG treatment, with Cl staying between 0.49 and 0.50%. In phase 3, it became even more difficult to limit Na content with increasing MSG; Na levels were 0.28% in the first 3 treatments and 0.34 and 0.44 in the highest MSG treatments, with Cl at 0.48% in all treatments. Thus, in Exp. 2, Na was controlled with the use of Na bicarbonate and KCl, and it was only in excess in a few of the highest MSG treatments. In this experiment, Cl was only marginally in excess.

Mahan et al. (1996) reported increasing growth performance, particularly in the first 7-d postweaning when supplementing pigs with NaCl, Na phosphate, or HCl in diets containing dried whey. A second study by Mahan et al. (1999) reported improved nursery pig performance and N retention and digestibility in diets containing spray dried animal plasma supplemented with Na and even more importantly, Cl up to 0.40%.

It appears the relationship between Na and Cl may be important, especially when adding MSG to the diet. Poor growth performance in Exp. 1 could be attributed to excessive Na in relation to Cl, particularly in the high salt treatment. When both Na and Cl levels were maintained in Exp. 2, performance was then similar across treatments.

In conclusion, while previous research has shown beneficial effects of added glutamate in nursery pig diets, our data did not confirm these findings via supplementation of MSG. This may be due to the dietary Na also contributed by MSG and importance of monitoring dietary Na and Cl in nursery pigs. Further research should continue to investigate how different glutamate

sources may interact with dietary Na and Cl levels and seek to understand their relationship in the gastrointestinal tract.

Literature Cited

- AOAC International. 2012. Official Methods of Analysis of AOAC Int. 19rd ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.
- Burrin, D. G., and B. Stoll. 2009. Metabolic fate and function of dietary glutamate in the gut. *Am. J. Clin. Nutr.* 90: 850S-856S. doi: 10.3945/ajcn.2009.27462Y
- Lin, M., B. Zhang, C. Yu, J. Li, L. Zhang, H. Sun, F. Gao, and G. Zhou. 2014. L-glutamate supplementation improves small intestinal architecture and enhances the expressions of jejunal mucosa amino acid receptors and transporters in weaning piglets. *PloS One.* 9(11): e111950. doi: 10.1371/journal.pone.0111950
- Liu, T., J. Peng, Y. Xiong, S. Zhou, and X. Cheng. 2002. Effects of dietary glutamine and glutamate supplementation on small intestinal structure, active absorption and DNA, RNA concentrations in skeletal muscle tissue of weaned piglets during d 28 to 42 of age. *Asian Australasian Journal of Animal Sciences.* 15: 238-242. doi: 10.5713/ajas.2002.238
- Mahan, D. C., E. A. Newton, and K. R. Cera. 1996. Effect of supplemental sodium chloride, sodium phosphate, or hydrochloric acid in starter pig diets containing dried whey. *J. Anim. Sci.* 74: 1217-1222.
- Mahan, D. C., T. D. Wiseman, E. Weaver, and L. Russell. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. *J. Anim. Sci.* 77: 3016-3021.
- NRC. 2005. Mineral Tolerance of Animals. 2nd rev. ed. Natl. Acad. Press, Washington, DC.
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

- Reeds, P. J., D. G. Burrin, B. Stoll, and F. Jahoor. 2000. Intestinal glutamate metabolism. *J. Nutr.* 130(4S Suppl):978S-82S.
- Rezaei, R., D. A. Knabe, C. D. Tekwe, S. Dahanayaka, M. D. Ficken, S. E. Fielder, S. J. Eide, S. L. Lovering, and G. Wu. 2013. Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. *Amino Acids.* 44:911–923. doi: 10.1007/s00726-012-1420-x
- Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for determination of fiber and lignin. *J. Assoc. Off. Anal. Chem.*, 46:829-835.
- Watford, M. 2008. Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. *J. Nutr.* 138(10): 2003S-2007S. doi: 138/10S-I/2003S
- Wu, G. 2014. Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *J. Anim. Sci. Biotechnol.* 5:34. doi: 10.1186/2049-1891-5-34
- Wu, G., and D. A. Knabe. 1994. Free and protein-bound amino acids in sow's colostrum and milk. *J. Nutr.* 124: 415-424.
- Wu, X., Y. Zhang, Z. Liu, T. J. Li, and Y. L. Yin. 2012. Effects of oral supplementation with glutamate or combination of glutamate and N-carbamylglutamate on intestinal mouse morphology and epithelium cell proliferation in weanling piglets. *J. Anim. Sci.* 90:337-339. doi: 10.2527/jas53752
- Zhang, J., Y. Yin, X. G. Shu, T. Li, F. Li, B. Tan, Z. Wu, and G. Wu. 2013. Oral administration of MSG increases expression of glutamate receptors and transporters in the gastrointestinal tract of young piglets. *Amino Acids.* 45:1169-1177. doi: 10.1007/s00726-013-1573-2

Table 1-1. Phase 1 diet composition (as-fed basis), Exp. 1¹

Ingredient, %	Monosodium glutamate ² , %					High Salt
	0	0.5	1.0	1.5	2.0	
Corn	39.37	38.84	38.30	37.76	37.22	38.86
Soybean meal, 48% CP	17.65	17.69	17.73	17.77	17.80	17.69
Corn DDGS, 6-9% Oil	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	4.50	4.50	4.50	4.50	4.50	4.50
HP 300 ³	2.50	2.50	2.50	2.50	2.50	2.50
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00
Choice white grease	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium P, 22% P	0.40	0.40	0.40	0.40	0.40	0.40
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30	0.78
L-Lys HCl	0.48	0.48	0.48	0.48	0.48	0.48
L-Thr	0.18	0.18	0.18	0.18	0.18	0.18
L-Trp	0.05	0.05	0.05	0.05	0.05	0.05
L-Val	0.10	0.10	0.10	0.10	0.10	0.10
Methionine hydroxy analog	0.24	0.24	0.24	0.24	0.24	0.24
Choline chloride, 60%	0.04	0.04	0.04	0.04	0.04	0.04
Phytase ³	0.04	0.04	0.04	0.04	0.04	0.04
Zinc oxide	0.39	0.39	0.39	0.39	0.39	0.39
Vitamin E, 20,000 IU	0.05	0.05	0.05	0.05	0.05	0.05
Selenium, 0.06%	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix ⁴	0.13	0.13	0.13	0.13	0.13	0.13
Vitamin premix ⁵	0.05	0.05	0.05	0.05	0.05	0.05
MSG	---	0.50	1.00	1.50	2.00	---
	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestibility (SID) amino acids, %						
Lys	1.40	1.40	1.40	1.40	1.40	1.40
Ile:Lys	55	55	55	55	55	55
Leu:Lys	111	111	111	111	110	111
Met:Lys	36	36	36	36	36	36
Met & Cys: Lys	56	56	56	56	56	56
Thr:Lys	62	62	62	62	62	62
Trp:Lys	19.1	19.1	19.1	19.1	19.1	19.1
Val:Lys	67	67	67	67	66	67
Total Lys, %	1.55	1.55	1.55	1.55	1.55	1.55
ME, kcal/kg	3,486	3,466	3,450	3,433	3,415	3,468
NE, kcal/kg	2,630	2,615	2,601	2,588	2,575	2,617
SID Lys:ME, g/Mcal	4.02	4.04	4.06	4.08	4.10	4.04
CP, %	21.0	21.0	20.9	20.9	20.9	21.0
Ca, %	0.71	0.71	0.71	0.71	0.71	0.71
P, %	0.67	0.67	0.67	0.66	0.66	0.67

Available P, %	0.59	0.59	0.59	0.59	0.59	0.59
Na, %	0.39	0.49	0.58	0.68	0.78	0.58
Cl, %	0.78	0.78	0.78	0.78	0.78	1.06

¹ Phase 1 was fed from d 0 to 12 in pelleted form.

² Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

³ Quantum Blue 5G (AB Vista, Plantation, FL) provided 2,000 phytase units (FTU)/kg of diet, for an estimated release of 0.15% available P.

⁴ Provided per kg of premix: 54 g Mn from manganese oxide, 27 g Mn from manganese oxide, 134 g Fe from iron sulfate, 160 g Zn from zinc sulphate, 13 g Cu from copper sulfate, 1370 mg I from calcium iodate.

⁵ Provided per kg of premix: 24,251 IU vitamin A; 4,409 IU vitamin D3; 132,277 IU vitamin E; 13,228 mg vitamin K; 17,637 mg riboflavin; 90,389 mg pantothenic acid; 99,208 mg niacin; 110 mg vitamin B12, 2,646 mg folic acid; 2,002 mg thiamin; 5,512 mg pyridoxine; and 441 mg biotin.

Table 1-2. Phase 2 and 3 diet composition (as-fed basis), Exp. 1¹

Ingredient, %	Phase 2			Phase 3		
	MSG ² , %		High Salt	MSG, %		High Salt
	0	2.0		0	2.0	
Corn	43.49	41.34	42.97	50.37	48.22	49.84
Soybean meal, 48% CP	22.42	22.57	22.45	24.79	24.94	24.83
Distillers dried grains with solubles	15.00	15.00	15.00	20.00	20.00	20.00
Fish meal	5.00	5.00	5.00	---	---	---
Dried whey	10.00	10.00	10.00	---	---	---
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.85	0.85	0.85	1.10	1.10	1.10
Monocalcium phosphate, 22% P	0.30	0.30	0.30	0.70	0.70	0.70
Salt	0.35	0.35	0.84	0.35	0.35	0.84
L-Lys HCl	0.40	0.40	0.40	0.50	0.50	0.50
DL-Met	0.14	0.14	0.14	0.13	0.13	0.13
L-Thr	0.13	0.13	0.13	0.15	0.15	0.15
L-Trp	0.03	0.03	0.03	0.03	0.03	0.03
Phytase ³	0.03	0.03	0.03	0.03	0.03	0.03
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
MSG	--	2.00	--	--	2.00	--
Trace mineral premix ⁴	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix ⁵	0.13	0.13	0.13	0.13	0.13	0.13
Antibiotics ⁶	0.40	0.40	0.40	0.40	0.40	0.40
	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestibility (SID) amino acids, %						
Lys	1.35	1.35	1.35	1.25	1.25	1.25
Ile:Lys	60	60	60	60	60	60
Leu:Lys	132	131	132	141	139	140
Met:Lys	37	37	37	35	35	35
Met & Cys:Lys	58	58	58	58	58	58
Thr:Lys	63	63	63	63	63	63
Trp:Lys	18.1	18.1	18.1	18.1	18.1	18.1
Val:Lys	68	67	68	68	67	68
Total Lys, %	1.54	1.54	1.54	1.43	1.43	1.43
ME, kcal/kg	3,360	3,291	3,342	3,327	3,258	3,311
NE, kcal/kg	2,469	2,414	2,456	2,434	2,381	2,421
SID Lys:ME, g/Mcal	4.02	4.10	4.04	3.76	3.84	3.78
CP, %	23.3	23.2	23.3	22.1	22.0	22.1
Ca, %	0.75	0.75	0.75	0.65	0.65	0.65
P, %	0.64	0.64	0.64	0.58	0.57	0.58
Available P, %	0.52	0.52	0.52	0.44	0.44	0.44
Na, %	0.31	0.69	0.50	0.23	0.61	0.42
Cl, %	0.62	0.62	0.91	0.54	0.54	0.83

¹ Phase 2 diets were fed from d 12 to 26 and phase 3 diets were fed from d 26 to 48.

² Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

³ Optiphos 2000, (Huvepharma Inc., Peachtree City, GA) provided 500 phytase units (FTU)/kg of diet, for an estimated release of 0.14% available P.

⁴ Provided per kg of premix: 33 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc sulphate, 17 g Cu from copper sulfate, 330 mg I from calcium iodate, and 300 mg Se from sodium selenite.

⁵ Provided per kg of premix: 7,054,674 IU vitamin A; 1,102,293 IU vitamin D3; 35,273 IU vitamin E; 3,527 mg vitamin K; 6,173 mg riboflavin; 22,046 mg pantothenic acid; 39,683 mg niacin; and 26 mg vitamin B12.

⁶ Denegard 10 (Elanco Animal Health, Greenfield, IN) providing tiamulin at 22 g/kg and Aureomycin 90 providing tetracycline at 198 g/kg (Zoetis, Florham Park, NJ).

Table 1-3. Phase 1 diet composition (as-fed basis), Exp. 2¹

Ingredient, %	Monosodium glutamate ² , %				
	0	0.5	1.0	1.5	2.0
Corn	34.23	34.08	33.88	33.63	33.06
Soybean meal, 48% CP	26.10	26.10	26.15	26.15	26.15
Fish meal	4.50	4.50	4.50	4.50	4.50
Corn DDGS	5.00	5.00	5.00	5.00	5.00
Lactose	20.00	20.00	20.00	20.00	20.00
Tallow	3.00	3.00	3.00	3.00	3.00
HP 300 ³	2.50	2.50	2.50	2.50	2.50
Monocalcium phosphate, 21% P	0.82	0.82	0.82	0.82	0.83
Limestone	0.72	0.72	0.72	0.72	0.72
Sodium bicarbonate	0.98	0.63	0.28	---	---
Potassium chloride	0.10	0.10	0.10	0.17	0.48
Zinc oxide	0.40	0.40	0.40	0.40	0.40
Salt	0.30	0.30	0.30	0.25	---
L-Lys HCl	0.48	0.48	0.48	0.48	0.48
DL-Met	0.23	0.23	0.23	0.23	0.24
L-Thr	0.25	0.25	0.25	0.25	0.25
L-Trp	0.06	0.06	0.06	0.06	0.06
L-Val	0.10	0.10	0.10	0.10	0.10
Choline chloride, 70%	0.04	0.04	0.04	0.04	0.04
Phytase ⁴	0.01	0.01	0.01	0.01	0.01
Vitamin E, 20,000 IU	0.05	0.05	0.05	0.05	0.05
Selenium, 0.06%	0.02	0.02	0.02	0.02	0.02
Vitamin and mineral premix ⁵	0.14	0.14	0.14	0.14	0.14
MSG	---	0.50	1.00	1.50	2.00
	100	100	100	100	100
Calculated Analysis					
Standardized ileal digestibility (SID)amino acids, %					
Lys	1.40	1.40	1.40	1.40	1.40
Met:Lys	39	39	39	39	39
Met & Cys:Lys	57	57	57	57	57
Thr:Lys	65	65	65	65	65
Trp:Lys	20	20	20	20	20
Val:Lys	67	67	67	67	67
Total Lys, %	1.56	1.56	1.57	1.56	1.56
ME, kcal/kg	3,458	3,453	3,448	3,439	3,420
CP, %	21.4	21.4	21.4	21.4	21.3
Ca, %	0.72	0.72	0.72	0.72	0.72
P, %	0.63	0.63	0.63	0.63	0.63
Available P, %	0.39	0.39	0.39	0.39	0.39
Na, %	0.43	0.43	0.43	0.43	0.43
Cl, %	0.50	0.50	0.50	0.50	0.50

¹ Phase 1 was fed from d 0 to 14 in pelleted form.

² Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

³ HP 300 (Hamlet Protein, Findlay, OH)

⁴ Optiphos 4000 PF (Huvepharma Inc., Peachtree City, GA) provided 401 phytase units (FTU)/kg of diet, with a release of 0.12% available P.

⁵ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 1-4. Phase 2 diet composition (as-fed basis), Exp. 2¹

Ingredient, %	Monosodium glutamate ² , %				
	0	0.5	1.0	1.5	2.0
Corn	47.84	47.69	47.32	46.82	46.31
Soybean meal, 48% CP	27.50	27.50	27.50	27.50	27.50
Fish meal	5.00	5.00	5.00	5.00	5.00
Corn DDGS	5.00	5.00	5.00	5.00	5.00
Lactose	10.00	10.00	10.00	10.00	10.00
Tallow	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate, 21% P	0.53	0.54	0.54	0.55	0.55
Limestone	0.83	0.83	0.83	0.83	0.83
Sodium bicarbonate	0.60	0.25	0.08	---	---
Potassium chloride	---	---	0.17	0.43	0.45
Zinc oxide	0.26	0.26	0.26	0.26	0.26
Salt	0.36	0.36	0.23	0.03	---
L-Lys HCl	0.40	0.40	0.40	0.40	0.40
DL-Met	0.18	0.18	0.18	0.18	0.19
L-Thr	0.18	0.18	0.18	0.18	0.18
L-Trp	0.02	0.02	0.02	0.02	0.03
L-Val	0.04	0.04	0.05	0.05	0.05
Choline chloride, 70%	0.04	0.04	0.04	0.04	0.04
Phytase ³	0.01	0.01	0.01	0.01	0.01
Vitamin E, 20,000 IU	0.05	0.05	0.05	0.05	0.05
Selenium, 0.06%	0.02	0.02	0.02	0.02	0.02
Vitamin and mineral premix ⁴	0.14	0.14	0.14	0.14	0.14
MSG ⁴	---	0.50	1.00	1.50	2.00
	100	100	100	100	100
Calculated Analysis					
Standardized ileal digestibility (SID) amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Met: Lys	38	38	38	38	38
Met & Cys: Lys	58	58	58	58	58
Thr: Lys	63	63	63	63	63
Trp: Lys	18	18	18	18	18
Val: Lys	67	67	67	67	67
Total Lys, %	1.52	1.52	1.52	1.52	1.52
ME, kcal/kg	3,343	3,338	3,326	3,309	3,292
CP, %	22.0	22.0	22.0	21.9	21.9
Ca, %	0.74	0.74	0.74	0.74	0.74
P, %	0.61	0.61	0.61	0.61	0.61
Available P, %	0.34	0.34	0.34	0.34	0.34
Na, %	0.35	0.35	0.35	0.35	0.43
Cl, %	0.49	0.49	0.49	0.50	0.49

¹ Phase 2 was fed from d 14 to 28 in meal form.² Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

³ Optiphos 4000 PF (Huvepharma Inc., Peachtree City, GA) provided 401 phytase units (FTU)/kg of diet, with a release of 0.12% available P.

⁴ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 1-5. Phase 3 diet composition (as-fed basis), Exp. 2¹

Ingredient, %	Monosodium glutamate ² , %				
	0	0.5	1.0	1.5	2.0
Corn	61.33	61.09	60.51	59.99	59.43
Soybean meal (48% CP)	28.65	28.65	28.65	28.70	28.75
Corn DDGS	5.00	5.00	5.00	5.00	5.00
Tallow	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate (21% P)	0.88	0.88	0.89	0.89	0.90
Limestone	1.12	1.12	1.13	1.13	1.13
Sodium bicarbonate	0.33	0.05	0.05	---	---
Potassium chloride	---	0.05	0.36	0.44	0.44
Zinc oxide	0.26	0.26	0.26	0.26	0.26
Salt	0.35	0.31	0.06	---	---
L-Lys HCl	0.45	0.45	0.45	0.45	0.45
DL-Met	0.15	0.15	0.16	0.16	0.16
L-Thr	0.17	0.17	0.17	0.17	0.17
L-Trp	0.02	0.02	0.02	0.02	0.02
L-Val	0.04	0.04	0.04	0.04	0.04
Choline chloride, 70%	0.04	0.04	0.04	0.04	0.04
Phytase ³	0.01	0.01	0.01	0.01	0.01
Vitamin E, 20,000 IU	0.05	0.05	0.05	0.05	0.05
Selenium, 0.06%	0.02	0.02	0.02	0.02	0.02
Vitamin and mineral premix ⁴	0.14	0.14	0.14	0.14	0.14
MSG	---	0.50	1.00	1.50	2.00
	100	100	100	100	100
Calculated Analysis					
Standardized ileal digestibility (SID)amino acids, %					
Lys	1.25	1.25	1.25	1.25	1.25
Met: Lys	35	35	35	35	35
Met & Cys:Lys	57	57	57	57	57
Thr:Lys	63	63	63	63	63
Trp:Lys	18	18	18	18	18
Val:Lys	67	67	67	67	67
Total Lys, %	1.40	1.40	1.40	1.40	1.40
ME, kcal/kg	3,276	3,268	3,248	3,232	3,215
CP, %	20.5	20.5	20.5	20.5	20.4
Ca, %	0.70	0.70	0.70	0.70	0.70
P, %	0.58	0.58	0.58	0.58	0.58
Available P, %	0.28	0.28	0.28	0.28	0.28
Na, %	0.28	0.28	0.28	0.34	0.44
Cl, %	0.48	0.48	0.48	0.48	0.48

¹ Phase 3 was fed from d 28 to 42 in meal form.

² Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

³ Optiphos 4000 PF (Huvepharma Inc., Peachtree City, GA) provided 401 phytase units (FTU)/kg of diet, with a release of 0.12% available P.

⁴ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; 198 mg Se from sodium selenite; 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 1-6. Chemical analysis of diets (as-fed basis), Exp. 1

Item	Monosodium Glutamate ¹ , %					
	0	0.5	1.0	1.5	2.0	High Salt
Phase 1 ²						
DM	92.28	92.57	92.25	92.55	92.35	92.77
CP	21.00	21.45	20.50	21.20	21.40	20.50
ADF	2.65	2.75	2.10	2.50	3.50	3.25
NDF	10.80	9.55	10.30	11.15	12.20	11.90
Crude fiber	2.10	1.70	1.80	1.90	2.05	1.90
Ca	0.90	0.93	0.77	0.83	0.75	0.80
P	0.72	0.73	0.67	0.68	0.66	0.69
Ether extract	5.95	5.90	6.30	6.05	6.30	6.25
Starch	24.70	23.10	25.25	25.05	24.30	25.25
Na	0.43	0.54	0.44	0.47	0.52	0.47
Cl	0.74	0.80	0.68	0.69	0.74	0.99
Phase 2 ³						
DM	89.88	89.09	89.76	89.46	89.94	89.65
CP	22.55	21.85	22.55	22.30	22.65	20.15
ADF	4.10	4.05	4.75	4.50	4.45	4.90
NDF	12.50	12.70	13.45	12.40	12.20	15.60
Crude fiber	2.80	2.70	3.25	3.00	3.30	3.55
Ca	1.07	1.04	0.93	0.96	0.87	0.97
P	0.73	0.73	0.74	0.77	0.76	0.77
Ether extract	4.50	4.90	4.65	4.70	4.90	5.45
Starch	27.40	27.30	27.05	26.25	25.15	26.60
Na	0.29	0.38	0.42	0.43	0.56	0.50
Cl	0.53	0.63	0.62	0.61	0.65	0.90
Phase 3 ⁴						
DM	88.64	88.62	88.59	89.51	89.25	89.24
CP	20.85	21.55	22.60	23.15	22.40	22.70
ADF	4.55	4.75	5.05	5.25	4.55	4.90
NDF	13.75	14.00	15.80	14.30	14.00	14.50
Crude fiber	3.05	3.30	3.30	3.45	3.10	3.25
Ca	0.78	0.76	0.77	0.69	0.89	0.81
P	0.62	0.63	0.67	0.64	0.67	0.68
Ether extract	4.10	4.20	4.25	4.25	4.05	3.70
Starch	31.90	31.45	29.85	31.25	30.35	29.95
Na	0.22	0.31	0.33	0.38	0.39	0.35
Cl	0.44	0.47	0.55	0.44	0.36	0.67

¹ Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

² Phase 1 was fed from d 0 to 12 in pelleted form.

³ Phase 2 was fed from d 12 to 26 in meal form. Diets with 0 and 2.0% MSG were manufactured and then blended to create the intermediate MSG treatments using a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN).

⁴ Phase 3 was fed from d 26 to 48 in meal form. Diets with 0 and 2.0% MSG were also blended to create the intermediate MSG treatments using a robotic feeding system.

Table 1-7. Chemical analysis of diets (as-fed basis), Exp. 2

Item, %	Monosodium glutamate ¹ , %				
	0	0.5	1.0	1.5	2.0
Phase 1 ²					
DM	88.9	90.45	89.02	90.05	90.14
CP	20.9	20.4	20.6	20.8	21.3
ADF	4.7	4.6	4.3	4.2	4.3
NDF	8.9	8.3	8.8	8.3	8.2
Crude fiber	2.4	2.9	2.5	3.4	2.5
Ca	0.73	0.62	0.64	0.64	0.64
P	0.71	0.64	0.63	0.63	0.63
Ether extract	5.6	5.7	5.6	5.5	5.6
Starch	20.9	21.0	20.6	20.7	20.1
Na	0.51	0.42	0.37	0.34	0.30
Cl	0.45	0.43	0.43	0.42	0.45
Phase 2 ³					
DM	88.69	89.37	89.58	89.04	89.26
CP	21.1	21.4	22.2	21.4	21.4
ADF	3.6	4.4	4	4.1	4.4
NDF	9.2	10.0	9.3	8.0	10.6
Crude fiber	2.3	3.2	2.4	2.3	2.6
Ca	0.77	0.74	0.82	0.75	0.73
P	0.66	0.64	0.68	0.67	0.66
Ether extract	4.2	4.3	4.1	4.1	4.0
Starch	28.1	28.2	27.1	28.5	27.0
Na	0.43	0.39	0.41	0.32	0.36
Cl	0.46	0.44	0.50	0.46	0.45
Phase 3 ⁴					
DM	87.71	87.72	87.89	87.21	87.27
CP	18.70	18.40	19.60	19.90	20.40
ADF	4.5	4.2	3.9	4.2	4.1
NDF	10.7	11.4	13.6	11.2	9.9
Crude fiber	2.80	2.70	2.40	2.80	2.70
Ca	0.61	0.64	0.59	0.59	0.54
P	0.63	0.60	0.60	0.59	0.59
Ether extract	4.0	3.9	3.9	3.9	3.9
Starch	37.5	38.1	38.3	37.4	36.2
Na	0.32	0.31	0.20	0.25	0.30
Cl	0.48	0.49	0.41	0.39	0.42

¹ Monosodium glutamate (MSG), Ajinomoto Heartland, Inc., Chicago, IL.

² Phase 1 was fed from d 0 to 14 in pelleted form.

³ Phase 2 was fed from d 14 to 28 in meal form.

⁴ Phase 3 was fed from d 28 to 42 in meal form.

Table 1-8. Effects of monosodium glutamate on nursery pig performance, Exp. 1¹

Item	Monosodium glutamate ² , %						SEM	MSG, <i>P</i> <		
	0.0	0.5	1.0	1.5	2.0	High Salt ³		Linear	Quadratic	High salt vs. 1% MSG
Phase 1 (d 0 to 12)										
ADG, g	119	128	127	125	130	117	6.2	0.163	0.537	0.103
ADFI, g	219	220	222	222	224	224	4.0	0.203	0.943	0.620
G:F	0.539	0.585	0.571	0.561	0.581	0.520	0.0227	0.326	0.518	0.069
Phase 2 (d 12 to 26)										
ADG, g	339	315	317	301	297	254	11.3	0.001	0.448	0.001
ADFI, g	508	460	470	482	460	445	16.0	0.045	0.221	0.123
G:F	0.668	0.684	0.674	0.627	0.645	0.572	0.0154	0.040	0.551	0.001
Phase 3 (d 26 to 48)										
ADG, g	575	546	561	548	551	528	10.1	0.173	0.347	0.028
ADFI, g	865	822	851	821	831	797	17.3	0.176	0.403	0.022
G:F	0.665	0.664	0.661	0.668	0.663	0.663	0.0084	0.962	0.933	0.843
Overall (d 0 to 48)										
ADG, g	388	372	378	369	370	341	7.9	0.033	0.340	0.001
ADFI, g	594	563	578	570	569	546	12.2	0.095	0.210	0.009
G:F	0.653	0.661	0.654	0.647	0.650	0.625	0.0044	0.132	0.606	0.001
BW, kg										
d 0	5.1	5.1	5.1	5.1	5.1	5.1	0.07	1.000	0.817	0.647
d 12	6.5	6.6	6.6	6.6	6.6	6.5	0.09	0.145	0.440	0.121
d 26	11.3	11.0	11.1	10.8	10.8	10.1	0.22	0.001	0.574	0.001
d 48	23.7	22.8	23.3	22.6	22.7	21.6	0.44	0.016	0.405	0.001

¹ A total of 1,134 nursery pigs (initially 5.1 kg BW) were used in a three phase nursery study with 27 pigs per pen and 7 replications per treatment.

² Treatments were determined according to increasing levels of monosodium glutamate (MSG, Ajinomoto Heartland, Inc., Chicago, IL.)

³ High salt treatment was formulated to match the Na content of the 1% MSG treatment.

Table 1-9. Effects of monosodium glutamate on nursery pig performance, Exp. 2^{1,2}

Item	Monosodium glutamate ³ , %					SEM	MSG, <i>P</i> <	
	0.0	0.5	1.0	1.5	2.0		Linear	Quadratic
Phase 1 (d 0 to 14)								
ADG, g	156	159	157	156	156	4.7	0.856	0.668
ADFI, g	187	194	191	191	189	4.2	0.992	0.284
G:F	0.831	0.821	0.823	0.821	0.828	0.0139	0.891	0.564
Phase 2 (d 14 to 28)								
ADG, g	528	521	513	524	524	8.0	0.824	0.220
ADFI, g	716	719	696	715	695	11.6	0.140	0.930
G:F	0.738	0.727	0.738	0.733	0.755	0.0081	0.065	0.059
Phase 3 (d 28 to 42)								
ADG, g	709	706	706	691	708	11.0	0.522	0.480
ADFI, g	1061	1071	1080	1057	1096	16.6	0.147	0.582
G:F	0.670	0.660	0.654	0.654	0.645	0.0057	0.003	0.689
Overall (d 0 to 42)								
ADG, g	464	462	458	457	461	5.3	0.538	0.438
ADFI, g	654	661	655	654	658	7.7	0.950	0.963
G:F	0.710	0.700	0.700	0.699	0.701	0.0037	0.119	0.080
BW, kg								
d 14	8.4	8.4	8.4	8.4	8.4	0.07	0.731	0.965
d 28	15.7	15.7	15.6	15.7	15.7	0.14	0.983	0.372
d 42	25.7	25.6	25.5	25.4	25.7	0.21	0.771	0.247

¹ A total of 700 nursery pigs (initially 6.2 kg BW) were used in a three phase nursery study with 10 pigs per pen and 14 replications per treatment.

² Initial BW was used as a covariate.

³ Treatments were determined according to increasing levels of monosodium glutamate (MSG, Ajinomoto Heartland, Inc., Chicago, IL.)

Chapter 2 - Modeling the effects of standardized ileal digestible valine to lysine ratio on growth performance of nursery pigs

Abstract

Two experiments evaluated the effects of increasing Lys and Val on nursery pig growth performance. In Exp. 1, 300 nursery pigs (PIC 327 × 1050, initially 6.7 ± 0.06 kg BW) were allotted to 1 of 6 diets containing 1.10, 1.20, 1.30, 1.40, 1.50, or 1.60% standardized ileal digestible (SID) Lys. There were 5 pigs per pen and 10 pens per treatment. Experimental diets were initiated 6-d post-weaning and fed for 14-d followed by a common diet from d 14 to 28. Diets were made by manufacturing the lowest and highest Lys basal diets and blending to create the intermediate treatments. Diets were formulated using NRC (2012) ingredient AA values and SID coefficients. From d 0 to 14, and the overall 28 d period, ADG and G:F increased (linear, $P < 0.001$) as SID Lys increased, with no differences in ADFI. Data were analyzed using heterogeneous variance where applicable and fitting 3 mixed models, quadratic polynomial (QP), broken-line linear (BLL), or broken-line quadratic (BLQ), selected for best-fit using Bayesian Information Criterion. For ADG and G:F, best-fitting models were the BLL and QP predicting maximum performance at 1.45% SID Lys and at least 1.60% SID Lys, respectively. In Exp. 2, 280 nursery pigs (PIC 327 × 1050, initially 6.5 ± 0.03 kg BW) were allotted to 1 of 7 diets containing SID Val:Lys ratios of 50, 57, 63, 68, 73, 78, or 85%. Dietary Lys concentration was set to be below the pig's requirement (1.24% SID Lys) to ensure accurate estimation of the Val:Lys ratio. There were 5 pigs per pen and 8 pens per treatment. Experimental diets were initiated 5-d post-weaning for 14 d followed by a common diet from d 14 to 28. The low and high SID Val:Lys ratio diets were

blended to form other treatments and were formulated using analyzed corn, soybean meal, and dried whey AA values and NRC (2012) SID AA coefficients. From d 0 to 14, ADG, ADFI, and G:F increased (quadratic, $P < 0.039$) with increasing SID Val:Lys ratio. For ADG, BLL predicted a minimum of 62.9% SID Val:Lys ratio, but for G:F, best fit model was QP reporting a maximum G:F at 71.7% SID Val:Lys ratio. Average daily feed intake was maximized at 73.7% Val:Lys. In conclusion, using the various statistical models presented, 1.45% SID Lys maximized ADG and G:F of 6- to 11- kg nursery pigs using BLL models while QP models predicted greater than 1.60%. The Val requirement ranged from 63 to 74% of Lys depending on the response criteria modeled.

Key words: amino acids, growth, lysine, nursery, pig, valine

Introduction

Amino acid requirement estimates are often expressed on a standardized ileal digestible (SID) basis and in ratios relative to Lys, typically the first limiting AA in corn and soybean meal diets for swine. While much is understood about the Lys requirement, it is important that it be determined prior to evaluating another limiting AA to encompass the specific genetics, environment, and BW of pigs. The NRC (2012) estimates the SID Lys requirement for 7- to 11- kg pigs is 1.35%. Wiltafsky et al. (2009), Gaines et al. (2011), and Nemechek et al. (2014) determined that a SID Val:Lys ratio of 65 to 67% was necessary for optimal growth of pigs ranging from 8 to 32 kg of BW. These values are often used as a recommendation for formulating diets with untested requirements.

When determining an AA requirement, advanced statistical methods may allow researchers to predict biological requirements with enhanced accuracy and precision. For example, Gonçalves et al. (2016a) detailed modeling strategies that account for

heterogeneity of residual variance, also known as heteroskedasticity. Heteroskedasticity seems to be a rather common phenomenon in animal agriculture (Cernicchiaro et al., 2013) and is characterized by unequal dispersion of residuals across groups of interest. Heteroskedasticity can be explicitly accommodated using a mixed modeling framework, which then translates into differential inferential precision across groups (Littell et al., 2006). This can be useful in the context of titration studies to characterize dose-response curves (Gonçalves et al., 2016a) to better predict nutrient requirements and consider performance as a response surface rather than single point requirement.

Therefore, the objective of these experiments was first to validate the Lys requirement and next, to determine the Val requirement for growth performance of 7- to 11- kg nursery pigs in a marginally Lys deficient scenario using a mixed modeling framework accounting for heteroskedasticity.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment.

General

Similar protocols were used in both experiments. Pigs (PIC 327 × 1050, Hendersonville, TN) were weaned at approximately 21 d of age, placed in nursery pens according to BW and gender, and fed a common pelleted starter diet for 6 (Exp. 1) or 5 d (Exp. 2). Then, pens of pigs were allotted to dietary treatments, and this was considered d 0 of the study. Each pen (1.52 × 1.52 m, Exp. 1; 1.52 × 1.22 m, Exp. 2) contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water. Both experiments were conducted at the Kansas State University Swine Teaching and

Research Center. Pigs and feeders were weighed on d 0, 14, and 28 to calculate ADG, ADFI, and G:F.

Dietary treatments were fed for 14 d followed by a common diet from d 14 to 28. Dietary treatments were corn- and soybean meal-based and contained 10% dried whey, fed in meal form. Crystalline AA replaced corn in diets as treatment levels of AA increased. During the common diet phase, diets were also corn- and soybean meal-based containing no specialty protein sources and formulated to 1.22% SID Lys. In Exp. 1, NRC (2012) ingredient nutrient values and SID AA coefficients were used in diet formulation. In Exp. 1, enzymatically treated soybean meal (HP 300, Hamlet Protein, Findlay, OH) was included at 10% and AA values and SID coefficients provided by the manufacturer were used. For Exp. 2, corn, soybean meal, and dried whey were analyzed for AA content prior to formulation (Table 2-1). All diets were fed in meal form and prepared at the O.H. Kruse Feed Technology and Innovation Center located in Manhattan, KS. For both experiments, basal diets were manufactured for the extreme treatments, and then blended at the feed mill to create the intermediate levels. Samples of experimental diets were submitted (Ward Laboratories, Kearney, NE) for analysis of DM (method 935.29; AOAC Int., 2012), crude fiber [method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY)], ash (method 942.05; AOAC Int., 2012), ether extract [method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY)], Ca and P [method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA)], Additional samples were submitted (Ajinomoto Heartland, Inc., Chicago, IL) for AA analysis (excluding Trp; method 994.12; AOAC

Int., 2012) and Trp (method 13904:2005; ISO, 2005) (Ajinomoto Heartland, Inc., Chicago, IL).

Experiment 1

A total of 300 nursery pigs (initially 6.7 ± 0.06 kg BW) were used to evaluate the effects of increasing SID Lys on growth performance. There were 10 pens per treatment and 5 pigs per pen. After 6 d in the nursery, pens of pigs were allotted to dietary treatments in a completely randomized design. The 6 dietary treatments were formulated to contain 1.10, 1.20, 1.30, 1.40, 1.50, and 1.60% SID Lys (Table 2-2). All other AAs exceeded estimated requirements on a ratio relative to Lys.

Experiment 2

A total of 280 nursery pigs (initially 6.5 ± 0.03 kg BW) were used to evaluate the effects of increasing SID Val:Lys ratio on growth performance. There were 8 pens per treatment and 5 pigs per pen. After 5 d in the nursery, pens were blocked by initial BW and then randomly assigned to dietary treatments in a randomized complete block design. The 7 dietary treatments were formulated to contain SID Val at 50, 57, 63, 68, 73, 78, and 85% of Lys (Table 2-3). Based on the results in Exp.1, experimental treatment diets were formulated to contain 1.24% SID Lys to ensure pigs were below their requirement.

Statistical Analysis

For each experiment, statistical analyses were performed as described by Gonçalves et al. (2016a). Briefly, preliminary analyses steps included fitting a base mixed model to data for each experiment, recognizing pen as the experimental unit and their respective designs (i.e. Exp.1: completely randomized design; Exp. 2: a randomized complete block design). Base models were fitted using the GLIMMIX procedure of SAS

(SAS Institute, Inc., 9.4, Cary, NC). For Exp. 1, there was a fixed effect of treatment and for Exp. 2, treatment was also fixed with block as a random effect. Results were considered significant at $P \leq 0.05$ and marginally significant at $P < 0.10$. For the base model, linear and quadratic contrasts were evaluated, with coefficients for unequal spacing between dietary treatments. Studentized residuals were evaluated for each response criteria. For Exp. 1, 1 pen on 1.30% SID Lys treatment and 1 pen on 1.40% SID Lys treatment were greater than 3 standard deviations from the means. These 2 pens were distinctly different from the remaining pens, and further investigation indicated that the outliers were due to extremely low growth rate not representative of the treatment means. Subsequently, data from these two pens was removed for all response criteria. The base model was also used to explore heterogeneity of residual variances across treatments for responses during the period when dietary treatments were fed using Bayesian Information Criteria (BIC) to decide on best fitting approaches to account for heteroskedasticity. After evaluation of the base model output in Exp. 1, there was no evidence of a dose relationship for ADFI. For Exp. 1, heterogeneous variance was applied only for G:F. For Exp. 2, heterogeneous variance was applied for ADG and ADFI, but not G:F. Initial BW was used as a covariate and block was removed from the ADFI models as it did not contribute to model fit. After evaluation of the base models, competing dose-response models were fit to ADG and G:F for Exp. 1 and ADG, ADFI, and G:F for Exp. 2 during the experimental period (d 0 to 14) using PROC GLIMMIX and PROC NLMIXED according to procedures of Gonçalves et al. (2016a) in order to characterize the functional forms of the relationship between each response and dietary treatments. The competing models fit were the quadratic polynomial (QP), broken-line linear (BLL), or broken-line

quadratic (BLQ), following Gonçalves et al. (2016a). The best-fitting dose-response model was decided using BIC, whereby a smaller BIC indicate a better fitting model; decreases of 2 points or more indicate decisive evidence for enhanced fit of the model with lower BIC (Raftery, 1996; Gonçalves et al., 2016a).

For the base model, treatment means were output along with SEM and covariate terms for Exp. 2. For dose-response models, BLL and BLQ output estimated breakpoints with respective 95% CI. For the QP model, the maximum response was output and the CI for the maximum response was calculated by plotting the regression equation across the dose levels with 95% CI. Then, the maximum estimated response is projected on the y-axis using a horizontal line and points of intersection of this line with the CI boundaries on the predicted line are then projected onto the x-axis as CI estimators of the optimum dose level (Gonçalves et al., 2016a).

Results and Discussion

Results of proximate and total AA analysis for both experiments closely matched formulated values (Tables 2-4 and 2-5). For Exp. 1, Lys content consistently increased across treatments. For Exp. 2, Val content increased in a step-wise manner and Lys remained constant.

In Exp. 1, from d 0 to 14, ADG and G:F increased (linear; $P < 0.001$) as SID Lys increased, with no evidence for differences in ADFI (Table 2-6). Furthermore, there was no evidence for differences in ADG, ADFI, or G:F during the common period (d 14 to 28). For the overall period (d 0 to 28), ADG and G:F increased (linear; $P < 0.001$) as SID Lys increased. Similarly, BW on d 14 and 28 also increased (linear; $P < 0.001$) with increasing SID Lys.

From d 0 to 14 when experimental diets were fed, evaluation of the base model resulted in the use of homogenous variance for ADG. For ADG (Figure 2-1), the best-fitting models were BLL and QP (BIC: 305.8 and 306.8, respectively). For the BLL, maximum ADG was achieved with a minimum of 1.45% SID Lys (95% CI: [1.31, 1.58%]). The QP resulted in a maximum ADG above 1.60% SID Lys (95% CI: [1.47, >1.60]) and 95% of maximum performance was achieved with 1.43% SID Lys.

The estimated regression equation for the BLL model was:

$$\text{ADG} = 319.66 - 176.65 \times (1.45 - \text{SID Lys}), \text{ when SID Lys} < 1.45 \%$$

$$\text{ADG} = 319.66, \text{ if SID Lys} \geq 1.45\%$$

where the SID Lys level is expressed as a percentage.

The estimated regression equation for the QP model was:

$$\text{ADG} = -183.1 + 586.6 \times (\text{SID Lys}) - 168.8 \times (\text{SID Lys})^2$$

Average daily feed intake used homogeneous variance for the base model, however, it showed no evidence of a dose-response relationship and thus, was not modeled further.

Feed efficiency for the experimental period (d 0 to 14), modeled with heterogeneous variance (Figure 2-2), had similar fitting models for the BLL and QP (BIC: 627.7 and 629.6, respectively). For the BLL, maximum G:F was achieved with a minimum of 1.45% SID Lys (95% CI: [1.35, 1.54%]). The QP reported maximum G:F above 1.60% SID Lys (95% CI: [1.53, >1.60]) and 95% of maximum performance was achieved with 1.41% SID Lys.

The estimated regression equation for the BLL model was:

$$\text{G:F} = 0.72657 - 0.35513 \times (1.45 - \text{SID Lys}), \text{ when SID Lys} < 1.45 \%$$

G:F = 0.72657, if SID Lys \geq 1.45%

The estimated regression equation for the QP model was:

$$G:F = -0.3041 + 1.2081 \times (\text{SID Lys}) - 0.3485 \times (\text{SID Lys})^2$$

The NRC (2012) estimates the SID Lys requirement for pigs weighing 7- to 11- kg to be 1.35%. The NRC (2012) requirement does not differentiate between different growth responses, specifically ADG and G:F. Researchers have shown that the requirement can be different for these growth responses, where Nemechek et al. (2012) determined that the SID Lys requirement for a 7- to 11- kg pig was 1.30 and 1.37% for ADG and G:F, respectively, using broken-line linear analysis. When using a quadratic broken-line analysis, the requirement increased to 1.37% and 1.54% SID Lys for ADG and G:F, respectively. In turn, Kendall et al. (2008) concluded that the true ileal digestible Lys requirement for 11- to 27- kg pigs was 1.30%. More recently, Park and Kim (2015) estimated the SID Lys requirement for 6- to 10- kg pigs to be 1.43% for ADG and ranging from 1.39 to 1.49% for G:F depending on alternative modeling strategies. These results are similar to data of the current study where it appears the requirement may be slightly greater than current NRC (2012) estimates. Our observed Lys requirement at levels greater than previously estimated may be due to modern genetics with potential for increased lean tissue accretion. Validating that the SID Lys requirement for 7- to 11- kg pigs in Exp. 1 was at least 1.45% allowed us to proceed with Exp. 2, whereby pigs were in a marginally Lys deficient setting, thus ensuring that the SID Val:Lys ratio requirement would not be underestimated.

In Exp. 2, during the experimental period (d 0 to 14), ADG, ADFI, and G:F increased (quadratic, $P < 0.039$) as SID Val:Lys ratio increased (Table 2-7). During the

common phase (d 14 to 28), ADFI increased and G:F decreased (linear, $P < 0.028$) in pigs previously fed diets containing increasing SID Val:Lys ratio. During the overall period (d 0 to 28), ADG marginally improved (quadratic, $P = 0.089$), while ADFI increased (linear, $P = 0.006$) as SID Val:Lys ratio from d 0 to 14 increased. Similarly, BW was initially increased and then decreased with increasing SID Val:Lys ratio (quadratic, $P = 0.001$) on d 14 and marginally increased (linear, $P = 0.057$) on d 28 when previously fed increasing SID Val:Lys.

For ADG (Figure 2-3), from d 0 to 14 when experimental diets were fed, heterogeneity of variance was applied and the BLL model showed the best fit with maximum ADG obtained with an estimated 62.9% SID Val:Lys ratio (95% CI: [52.2, 73.7%]).

The estimated regression equation for the BLL model was:

$$\text{ADG} = 247.021 - 4.383 \times (62.9 - \text{SID Val:Lys}), \text{ when SID Val:Lys} < 62.9 \%$$

$$\text{ADG} = 247.021, \text{ if SID Val:Lys} \geq 62.9\%$$

using a 6.5 kg initial BW and where Val is expressed as a percentage of Lys (i.e. 50%).

For ADFI (Figure 2-4), modeled with homogenous variance, the QP was the best fitting model, estimating maximum feed intake at 73.7% SID Val:Lys ratio [95% CI: (61, >85)] and 99% of maximum performance achieved with 68.0% SID Val:Lys ratio.

The prediction equation for the QP for a 6.5 kg pig is as follows:

$$\text{ADFI} = -253.297 + 17.6999 \times (\text{SID Val:Lys}) - 0.1201 \times (\text{SID Val:Lys})^2$$

For G:F (Figure 2-5), homogeneous variance was used and the best fitting model was the QP model, which yielded maximum G:F at an estimated 71.7% SID Val:Lys

ratio [95% CI: (58, >85)] and 99% of maximum performance achieved with 64.4% SID Val:Lys.

The prediction equation for a 6.5 kg pig for the G:F QP is as follows:

$$\text{G:F} = 0.010294 + 0.017526 \times (\text{SID Val:Lys}) - 0.000122 \times (\text{SID Val:Lys})^2$$

The lowest Val:Lys ratio used in this experiment was previously confirmed to be deficient for nursery pigs weighing 7- to 11- kg by Nemechek et al. (2014). We designed our dietary treatments to increase SID Val:Lys ratio from 50 to 85% to model dose-response and be able to estimate requirement points at which performance was maximized. The levels at which no further increase in performance was observed in the present experiment were 63, 74, and 72% SID Val:Lys ratio for ADG, ADFI, and G:F, respectively. Several other studies have observed similar ranges of requirement estimates (Gaines et al., 2011; Nemechek et al. 2014; Soumeh et al., 2015). Gaines et al. (2011) used single-slope broken-line methods in determining a SID Val:Lys ratio requirement estimate for 13- to 32- kg pigs and found that a ratio of 65% was sufficient for ADG and G:F. Barea et al. (2009) determined that the SID Val:Lys ratio requirement for pigs weighing approximately 12- to 25- kg post-weaned pigs was 70, 74, and 68% for ADG, ADFI, and G:F, respectively, using a linear-plateau model and 75, 81, and 68, respectively, using curvilinear-plateau models. Furthermore, Wiltafsky et al. (2009) estimated that the ideal SID Val:Lys ratio for 8- to 25- kg pigs was 65 to 67%. Nemechek et al. (2014) reported that 65% SID Val:Lys was adequate for optimal growth of 7- to 11- kg pigs. Using individually housed pigs weighing 8- to 14- kg, Soumeh et al. (2015) identified that SID Val needed to be 70% of Lys using linear and curvilinear models. Finally, the NRC (2012) suggests a 64% SID Val:Lys ratio for 7- to 11- kg nursery pigs.

An estimated range of SID Val:Lys ratio requirements is also available for heavier pigs. Waguespack et al. (2012) cite a 67 to 70% SID Val:Lys ratio requirement for 20- to 45- kg pigs. Additionally, when observing the Val:Lys ratio requirement for several weight ranges of pigs, Liu et al. (2015) cite the requirement of SID Val at 62, 66, 67, and 68% of Lys for 26- to 46- kg, 49- to 70- kg, 71- to 92- kg, and 94- to 199- kg pigs, respectively, using broken-line linear models. However, when using quadratic models, these requirements increased to 71, 72, 73, and 72% of Lys, respectively. These models sought to maximize ADG and minimize serum urea N. Lastly, Gonçalves et al. (2016b) evaluated the Val:Lys ratio requirement in a commercial research environment (25 pigs per pen) of pigs weighing 25- to 120- kg using the same modeling techniques as used in our studies and concluded that SID Val at 67% of Lys was sufficient to capture 99% of ADG and G:F.

The ability to apply subjective performance goals (i.e., 95 or 99% of maximum performance) allows nutritionists to determine an optimum AA level. These levels can vary depending on production system goals and economics. Providing requirements for all growth response criteria enables producers to, for instance, determine where 100% of maximum ADG can be captured, while still capturing 99% of another response. This will ultimately create the best scenario to optimize economic value in setting a dietary AA level. For example, formulating SID Val to 63% of Lys captures 100% of ADG performance, while also achieving 96.6% and 98.6% of ADFI and G:F performance, respectively. Thus, these response surfaces can be considered first individually and then as a whole in the decision making process.

It is important to note that the different statistical methods and models utilized in evaluating the data in various experiments could explain some variation in the results among studies. However, with the exception of the curvilinear-plateau models used by Barea et al. (2009), all other literature previously cited are in agreement with the range in the present data of 63 to 74% SID Val:Lys ratio. In conclusion, the NRC (2012) requirement for SID Val:Lys is similar to our results, while our data produce a range depending on the response criteria. Average daily gain produced the lowest estimate at 63% of Lys, while ADFI and G:F had greater estimates, indicating that the requirement may be variable depending on the response of interest.

Literature Cited

- Barea, R., L. Brossard, L. Floc'h, Y. Primot, D. Melchior, and J. Van Milgen. 2009. The standardized ileal digestible valine-to-lysine requirement ratio is at least seventy percent in postweaned piglets. *J. Anim. Sci.* 87:935-947. doi: 10.2527/jas.2008-1006
- Cernicchiaro, N., D. G. Renter, S. Xiang, B. J. White, and N. M. Bello. 2013. Hierarchical Bayesian modeling of heterogeneous variances in average daily weight gain of commercial feedlot cattle. *J. Anim. Sci.* 91:2910–2919. doi: 10.2527/jas.2012-5543
- Gaines, A., D. Kendall, G. Allee, J. Usry, and B. Kerr. 2011. Estimation of the standardized ileal digestible valine-to-lysine ratio in 13-to 32-kilogram pigs. *J. Anim. Sci.* 89:736-742. doi: 10.2527/jas.2010-3134
- Gonçalves, M. A. D., N. M. Bello, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, J. C. Woodworth, and R. D. Goodband. 2016a. An update on modeling dose–response relationships: Accounting for correlated data structure and heterogeneous error variance in linear and nonlinear mixed models. *J. Anim. Sci.* 94(5): 1940-1950. doi: 10.2527/jas2015-0106
- Gonçalves, M. A. D., M. D. Tokach, S. S. Dritz, N. M. Bello, K. J. Touchette, R. D. Goodband, J. M. DeRouchey, J. C. Woodworth. 2016b. Effects of standardized ileal digestible valine-to- lysine ratio on growth performance of twenty- five– to forty-five–kilogram pigs under commercial conditions. *J. Anim. Sci.* 94(Suppl. 2):19-20 (Abstr.).

- Kendall, D. C., A. M. Gaines, G. L. Allee, and J. L. Usry. 2008. Commercial validation of the true ileal digestible lysine requirement for eleven-to twenty-seven-kilogram pigs. *J. Anim. Sci.* 86:324-332. doi: 10.2527/jas.2007-0086
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS® for mixed models, 2nd ed. SAS Inst. Inc., Cary, NC.
- Liu, X. T., W. F. Ma, X. F. Zeng, C. Y. Xie, P. A. Thacker, J. K. Htoo, and S. Y. Qiao. 2015. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kilogram pigs fed low crude protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* 93:4761-4773. doi: 10.2527/jas2015-9308
- Nemecek, J. E., A. M. Gaines, M. D. Tokach, G. L. Allee, R. D. Goodband, J. M. DeRouche, J. L. Nelssen, J. L. Usry, G. Gourley, and S. Dritz. 2012. Evaluation of standardized ileal digestible lysine requirement of nursery pigs from seven to fourteen kilograms. *J. Anim. Sci.* 90:4380-4390. doi: 10.2527/jas.2011-5131
- Nemecek, J. E., M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. M. DeRouche. 2014. Evaluation of standardized ileal digestible valine: Lysine, total lysine: Crude protein, and replacing fish meal, meat and bone meal, and poultry byproduct meal with crystalline amino acids on growth performance of nursery pigs from seven to twelve kilograms. *J. Anim. Sci.* 92 548-1561. doi: 10.2527/jas2013-6322
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Park, C. S., and B. G. Kim. 2015. Standardized ileal digestible lysine requirement of 6-to 10-kg weanling pigs. *AJAVSP.* 10:150-155. doi: 10.3844/ajavsp.2015.150.155

- Raftery, A. E. 1996. Approximate Bayes factors and accounting for model uncertainty in generalized linear regression models. *Biometrika* 83:251–266. doi: 10.1093/biomet/83.2.251
- Soumeh, E. A., J. van Milgen, N. M. Sloth, E. Corrent, H. D. Poulsen, and J. V. Nørgaard. 2015. Requirement of standardized ileal digestible valine to lysine ratio for 8-to 14-kg pigs. *Animal*. 9(08): 1312-1318. doi: 10.1017/S1751731115000695
- Waguespack, A. M., T. D. Bidner, R. L. Payne, and L. L. Southern. 2012. Valine and isoleucine requirement of 20-to 45-kilogram pigs. *J. Anim. Sci.* 90:2276-2284. doi: 10.2527/jas2011-4454
- Wiltafsky, M. K., B. Schmidlein, and F. X. Roth. 2009. Estimates of the optimum dietary ratio of standardized ileal digestible valine to lysine for eight to twenty-five kilograms of body weight pigs. *J. Anim. Sci.* 87:2544-2553. doi: 10.2527/jas.2008-1221

Table 2-1. Chemical analysis of ingredients for Exp. 2^{1,2}

Item, %	Corn	Soybean meal	Dried whey
Total AA			
Lys	0.29	2.88	0.79
Ile	0.31	2.09	0.65
Leu	1.10	3.51	1.07
Met	0.18	0.66	0.16
Thr	0.30	1.80	0.68
Trp	0.07	0.65	0.22
Val	0.40	2.12	0.59
His	0.24	1.17	0.18
Phe	0.43	2.35	0.37
Standardized ileal digestible AA, % (Calculated)			
Lys	0.21	2.56	0.77
Ile	0.25	1.86	0.62
Leu	0.95	3.09	1.05
Met	0.15	0.59	0.16
Thr	0.23	1.53	0.61
Trp	0.06	0.59	0.21
Val	0.32	1.84	0.56
His	0.20	1.05	0.17
Phe	0.36	2.07	0.34

¹ Analyzed for AA content at Ajinomoto Heartland, Inc. (Chicago, IL).

² Standardized ileal digestible (SID) concentration calculated using SID coefficients from the NRC (NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.).

Table 2-2. Diet composition (as-fed basis), Exp. 1¹

Item	Formulated SID ² Lys, %		Common Phase
	1.10	1.60	
Ingredient, %			
Corn	59.06	48.15	63.77
Soybean meal, 48% CP	26.89	27.05	32.86
Dried whey	10.00	10.00	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate, 22% P	1.60	1.50	1.10
Salt	0.30	0.30	0.35
L-Lys-HCl	0.25	0.55	0.3
DL-Met	0.13	0.33	0.12
L-Thr	0.10	0.26	0.12
L-Trp	0.02	0.06	---
L-Val	0.01	0.15	---
Trace mineral premix ³	0.15	0.15	0.15
Vitamin premix ⁴	0.25	0.25	0.25
Zinc oxide	0.25	0.25	---
HP 300 ⁵	---	10.00	---
Total	100.00	100.00	100.00
Calculated analysis ⁶			
SID AA, %			
Lys	1.10	1.60	1.22
Ile:Lys	64	57	63
Leu:Lys	133	109	129
Met:Lys	35	40	33
Met & Cys:Lys	60	59	57
Thr:Lys	65	65	63
Trp:lys	20.4	20.3	18.7
Val:Lys	70	70	69
Total Lys, %	1.23	1.77	1.37
ME, kcal/kg	3,256	3,302	3,272
NE, kcal/kg	2,427	2,407	2,407
SID Lys:ME, g/Mcal	3.38	4.84	3.73
SID Lys:NE, g/Mcal	4.57	7.44	5.16
CP, %	19.3	24.7	21.4
Ca, %	0.82	0.83	0.70
P, %	0.76	0.79	0.64
Available P, %	0.48	0.48	0.41

¹ Treatments 1.10% and 1.60% SID Lys were manufactured and blended at the feed mill to create the intermediate levels of 1.20%, 1.30%, 1.40%, and 1.50% SID Lys.

² Standardized ileal digestible.

³ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

⁴ Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3;

17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

⁵Hamlet Protein, Findley, OH.

⁶NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

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

Item	Formulated SID ² Val:Lys Ratio, %		
	50	85	Common Phase
Ingredient, %			
Corn	62.97	62.50	63.77
Soybean meal, 48% CP	22.07	22.11	32.86
Dried whey	10.00	10.00	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate, 22% P	1.65	1.65	1.10
Salt	0.30	0.30	0.35
L-Lys-HCl	0.63	0.63	0.3
DL-Met	0.27	0.27	0.12
L-Thr	0.29	0.29	0.12
L-Trp	0.08	0.08	--
L-Val	0.00	0.44	--
L-Ile	0.10	0.10	--
Trace mineral premix ³	0.15	0.15	0.15
Vitamin premix ⁴	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00
Calculated analysis			
SID AA, %			
Lys	1.24	1.24	1.22
Ile:Lys	57	57	63
Leu:Lys	110	110	129
Met:Lys	40	40	33
Met & Cys:Lys	60	60	57
Thr:Lys	66	66	63
Trp:Lys	20.1	20.1	18.7
Val:Lys	50	85	69
Total Lys, %	1.36	1.36	1.37
ME, kcal/kg	3,289	3,298	3,272
NE, kcal/kg	2,427	2,407	2,407
SID Lys:ME, g/Mcal	3.75	3.74	3.73
SID Lys:NE, g/Mcal	5.09	5.08	5.16
CP, %	17.6	17.9	21.4
Ca, %	0.82	0.82	0.70
P, %	0.73	0.73	0.64
Available P, %	0.49	0.49	0.41

¹ The 50% and 85% SID Val:Lys diets were manufactured and blended at the feed mill to create the intermediate Val Concentrations at 57, 63, 68, 73, and 78% of Lys.

² Standardized ileal digestible.

³ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

⁴ Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 2-4. Chemical analysis of diets (as-fed basis), Exp. 1¹

Item:	Formulated standardized ileal digestible (SID) Lys, % ²					
	1.10	1.20	1.30	1.40	1.50	1.60
Item, % ³						
DM	88.77	88.24	88.81	87.35	89.18	89.22
CP	20.6	20.9	21.6	23.0	23.4	24.4
Crude fiber	1.8	1.7	2.1	1.9	1.9	2.2
Ether extract	2.5	2.2	2.4	2.4	2.3	2.4
Ash	5.05	5.58	5.31	5.60	5.81	5.52
AA analysis, % ⁴						
Lys	1.26	1.38	1.42	1.52	1.60	1.75
Ile	0.83	0.86	0.91	0.94	0.96	1.02
Leu	1.76	1.76	1.83	1.88	1.93	1.98
Met	0.40	0.47	0.48	0.51	0.54	0.65
Met + Cys	0.75	0.81	0.84	0.88	0.92	1.04
Thr	0.80	0.85	0.92	1.00	1.02	1.12
Trp	0.25	0.26	0.28	0.30	0.32	0.35
Val	0.91	0.95	1.03	1.08	1.12	1.22
His	0.49	0.52	0.52	0.56	0.58	0.60
Phe	0.95	0.98	1.03	1.06	1.11	1.15

¹ Diet samples were collected at the feed mill after manufacturing.

² Low (1.10% SID Lys) and high (1.60% SID Lys) diets were blended at the feed mill to create the intermediate treatments.

³ Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

⁴ Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for AA analysis.

Table 2-5. Chemical analysis of diets (as-fed basis), Exp. 2¹

Item:	Formulated standardized ileal digestible (SID) Val:Lys ratio, % ²						
	50	57	63	68	73	78	85
Item, % ³							
DM	89.84	90.16	90.37	90.24	90.35	90.06	90.24
CP	17.0	18.7	17.6	18.0	18.0	19.3	17.6
Crude fiber	2.0	1.7	1.7	1.7	1.2	2.0	1.8
Ether extract	2.6	2.2	2.2	2.4	2.2	2.3	2.2
Ash	5.25	5.58	5.26	5.08	5.17	5.14	5.14
AA analysis, % ⁴							
Lys	1.32	1.33	1.37	1.35	1.35	1.33	1.34
Ile	0.76	0.78	0.77	0.77	0.78	0.87	0.80
Leu	1.56	1.54	1.54	1.51	1.55	1.61	1.59
Met	0.46	0.50	0.50	0.48	0.49	0.46	0.48
Met + Cys	0.73	0.77	0.77	0.74	0.78	0.75	0.77
Thr	0.92	0.89	0.92	0.89	0.90	0.94	0.96
Trp	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Val	0.78	0.84	0.88	0.92	0.99	1.04	1.10
His	0.43	0.42	0.41	0.41	0.42	0.44	0.43
Phe	0.83	0.92	0.82	0.80	0.83	0.86	0.84

¹ Treatment diet samples were collected at the feed mill after manufacturing.

² Low (50% SID Val:Lys) and high (85% SID Val:Lys) diets were blended at the feed mill to create the intermediate treatments.

³ Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

⁴ Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for AA analysis.

Table 2-6. Effects of standardized ileal digestible (SID) Lys on nursery pig growth performance^{1,2}

Item:	Formulated SID Lys, % ³						SEM	Probability, <i>P</i> <	
	1.10	1.20	1.30	1.40	1.50	1.60		Linear	Quadratic
Treatment period (d 0 to 14)									
ADG, g	265	263	298	313	319	320	9.6 to 10.1	0.001	0.278
ADFI, g	432	417	446	438	441	442	13.9 to 14.6	0.336	0.835
G:F	0.616	0.631	0.670	0.714	0.725	0.729	0.0096 to 0.0188	0.001	0.249
Post-treatment period (d 14 to 28)									
ADG, g	565	568	580	554	578	582	12.6 to 13.3	0.391	0.653
ADFI, g	886	890	916	875	909	925	17.2 to 18.1	0.154	0.558
G:F ⁹	0.637	0.639	0.633	0.634	0.636	0.630	0.0086 to 0.0091	0.578	0.954
Overall (d 0 to 28)									
ADG, g	415	416	439	433	448	451	9.3 to 9.8	0.001	0.797
ADFI, g	659	653	681	657	675	683	14.1 to 14.9	0.180	0.799
G:F	0.630	0.636	0.645	0.661	0.665	0.661	0.0086 to 0.0091	0.001	0.401
BW, kg									
d 0	6.7	6.7	6.8	6.7	6.7	6.7	0.06	0.952	0.721
d 14	10.4	10.4	10.9	11.1	11.2	11.2	0.15	0.001	0.263
d 28	18.3	18.3	19.0	18.9	19.3	19.3	0.27 to 0.29	0.001	0.758

¹ A total of 300 nursery pigs (PIC 327 × 1050, initially 6.7 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then fed experimental diets.

² Experimental diets were fed from d 0 to 14 and a common diet was fed from d 14 to 28.

³ Low (1.10% SID Lys) and high (1.60% SID Lys) diets were blended upon manufacturing at the feed mill to create the 1.20, 1.30, 1.40, and 1.50% SID Lys dietary treatments.

Table 2-7. Effects of standardized ileal digestible (SID) Val:Lys ratio on nursery pig growth performance^{1,2}

Item:	Formulated SID Val:Lys, % ³							SEM	Probability, <i>P</i> <	
	50	57	63	68	73	78	85		Linear	Quadratic
Treatment period (d 0 to 14)										
ADG, g	190	221	249	249	248	251	238	5.9 to 11.9	0.001	0.001
ADFI, g	331	363	394	388	403	390	386	17.1 to 17.2	0.012	0.030
G:F	0.579	0.612	0.635	0.646	0.614	0.645	0.617	0.0188 to 0.0189	0.101	0.039
Post-treatment period (d 14 to 28)										
ADG, g	541	531	515	575	522	530	539	15.4 to 15.5	0.992	0.945
ADFI, g	826	817	825	878	847	866	876	22.9 to 23.0	0.028	0.965
G:F ⁹	0.654	0.651	0.624	0.655	0.616	0.612	0.616	0.0099 to 0.0100	0.001	0.923
Overall (d 0 to 28)										
ADG, g	366	376	382	412	385	391	389	11.0	0.067	0.089
ADFI, g	579	590	609	633	625	628	631	17.1 to 17.2	0.006	0.266
G:F	0.632	0.639	0.628	0.652	0.616	0.622	0.616	0.0105	0.104	0.303
BW, kg										
d 14	9.2	9.6	10.0	10.0	10.0	10.0	9.9	0.16	0.001	0.001
d 28	16.8	17.1	17.1	18.1	17.3	17.5	17.5	0.32	0.057	0.146

¹ A total of 280 nursery pigs (PIC 327 × 1050, initially 6.5 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then fed experimental diets. Initial (d 0) BW was used as a covariate.

² Experimental diets were fed from d 0 to 14 and a common diet was fed from d 14 to 28.

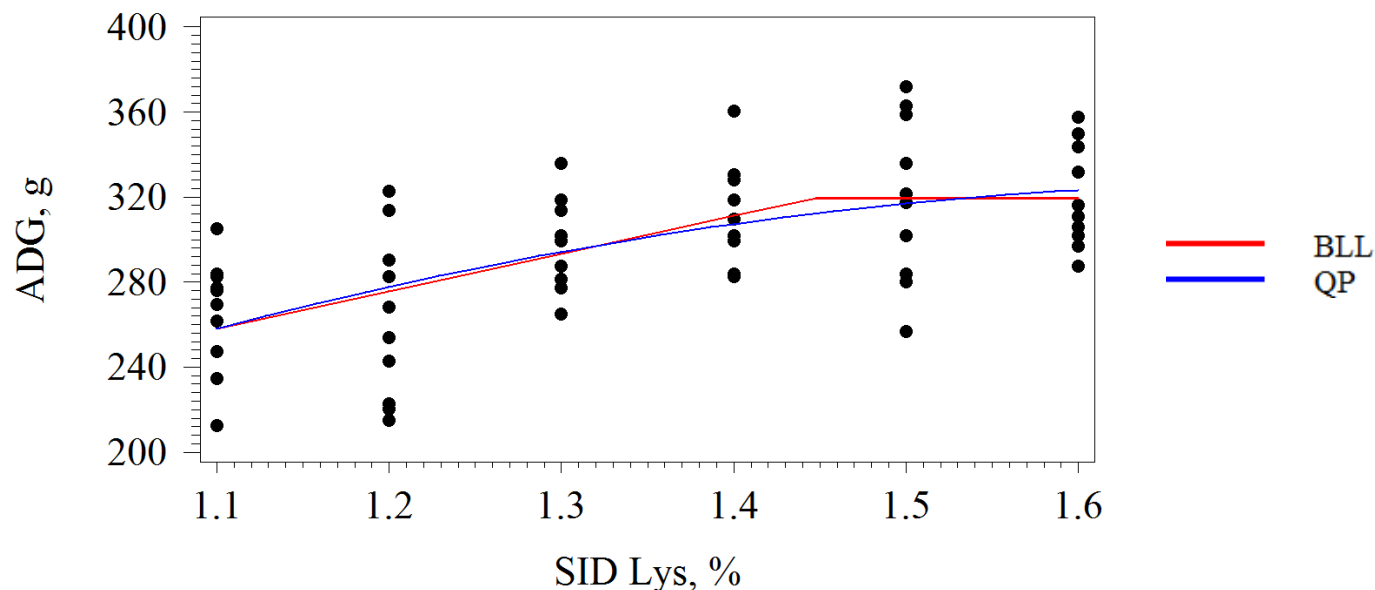
³ Low (50% SID Val:Lys ratio) and high (85% SID Val:Lys ratio) diets were blended upon manufacturing at the feed mill to create the 57, 63, 68, 73, and 78% SID Val:Lys ratio dietary treatments.

Figure 2-1. Estimated standardized ileal digestible (SID) Lys requirement to maximize ADG for nursery pigs¹

ADG

BLL 1.45% SID Lys, (95% CI: 1.31, 1.58);
 ADG = 319.66 – 176.65 × (1.45 – SID Lys), when SID
 Lys < 1.45 %
 ADG = 319.66, if SID Lys ≥ 1.45% (BIC =305.8)

QP Maximum: >1.60% SID Lys, (95% CI: 1.47, >1.60)
 95% Maximum: 1.43% SID Lys
 ADG = – 183.1 + 586.6 × (SID Lys) – 168.8 × (SID Lys)²
 (BIC = 306.8)



¹ A total of 300 nursery pigs (PIC 327 × 1050, initially 6.7 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then fed experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to characterize the SID Lys dose response curve. The BLL and QP models were the best fitting models based on Bayesian Information Criterion (BIC).

Figure 2-2. Estimated standardized ileal digestible (SID) Lys requirement to maximize feed efficiency (G:F) for nursery pigs¹

G:F

QP Maximum: >1.60% SID Lys, (95% CI: 1.53, >1.60)

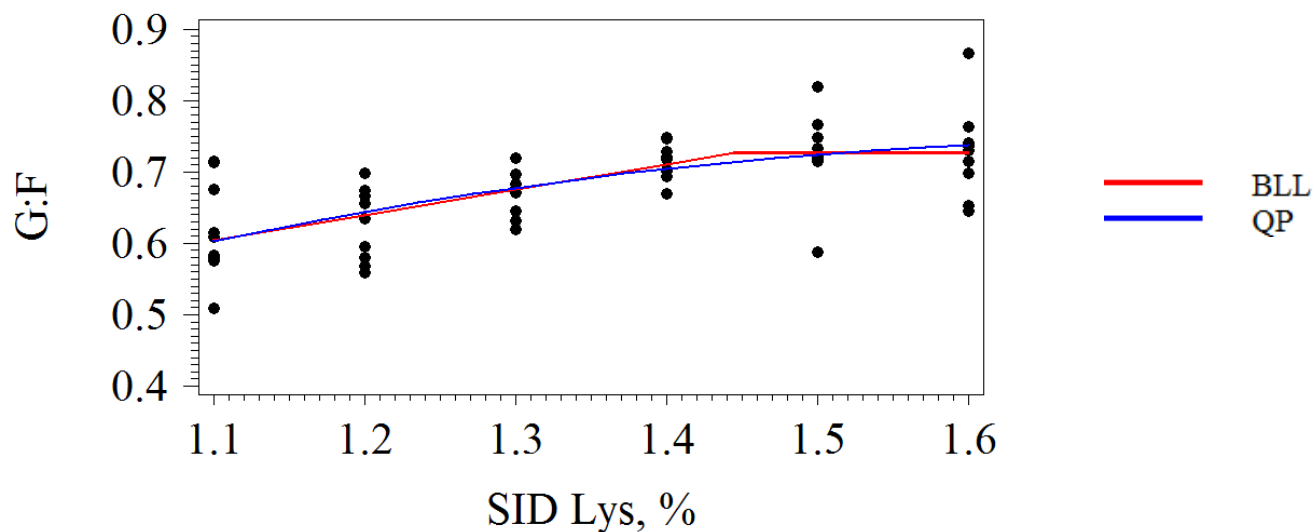
95% Maximum: 1.41% SID Lys

$G:F = -0.3041 + 1.2081 \times (\text{SID Lys}) - 0.3485 \times (\text{SID Lys})^2$
(BIC = 629.6)

BLL Breakpoint 1.45% SID Lys, (95% CI: 1.35, 1.54);

$G:F = 0.72657 - 0.35513 \times (1.45 - \text{SID Lys})$, when SID Lys < 1.45 %

$G:F = 0.72657$, if SID Lys \geq 1.45%
(BIC = 627.7)



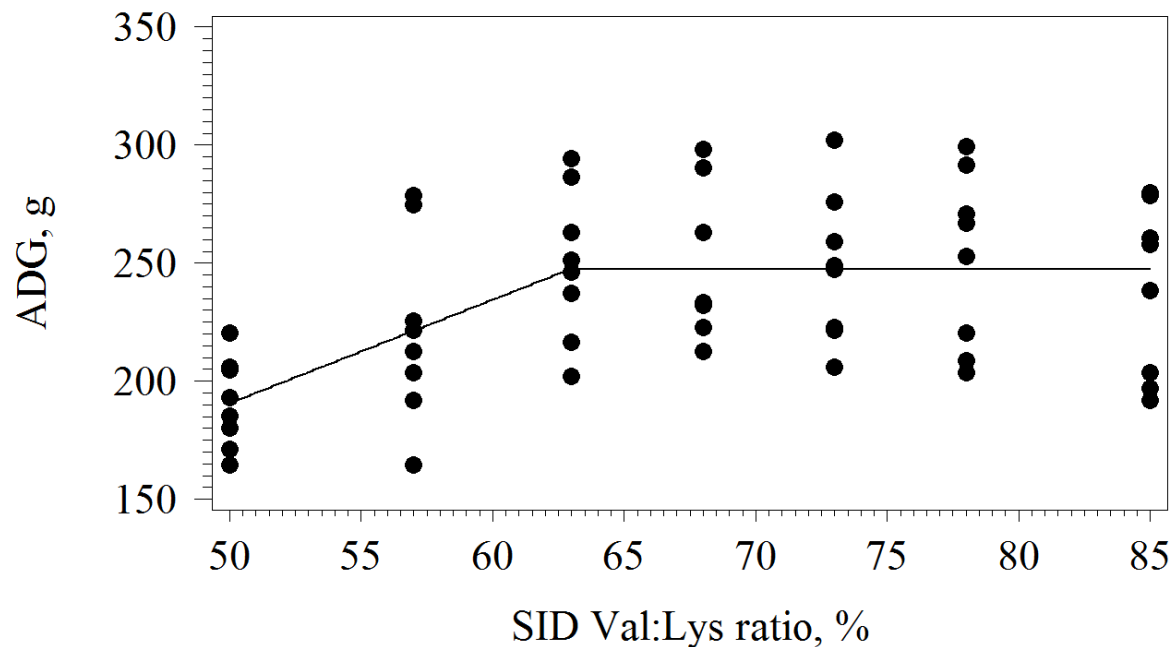
¹ A total of 300 nursery pigs (PIC 327 \times 1050, initially 6.7 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then fed experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to characterize the SID Lys dose response curve. The BLL and QP models were the best fitting models based on Bayesian Information Criterion (BIC).

Figure 2-3. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize ADG for nursery pigs¹

ADG BLL Breakpoint 62.9% SID Val:Lys, (95% CI: 52.2, 73.7);

ADG = 247.021 – 4.383 × (62.9 – SID Val:Lys), when SID Val:Lys < 62.9 %

ADG = 247.021, if SID Val:Lys ≥ 62.9%



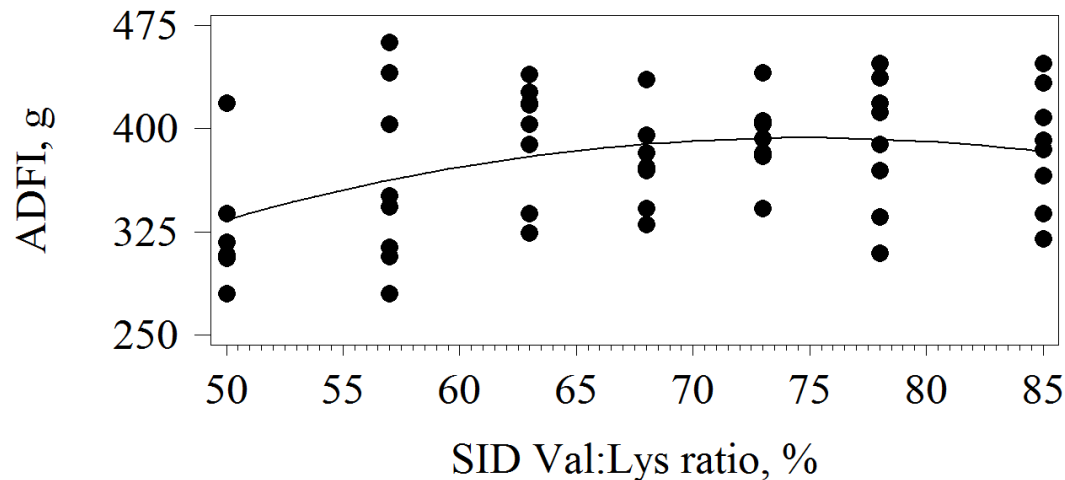
¹A total of 280 nursery pigs (PIC 327 × 1050, initially 6.5 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then fed experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys ratio level to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Figure 2-4. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize ADFI for nursery pigs¹

ADFI QP Maximum: 73.7% SID Val:Lys, (95% CI: 61, >85)

99% Maximum: 68.0% SID Val:Lys

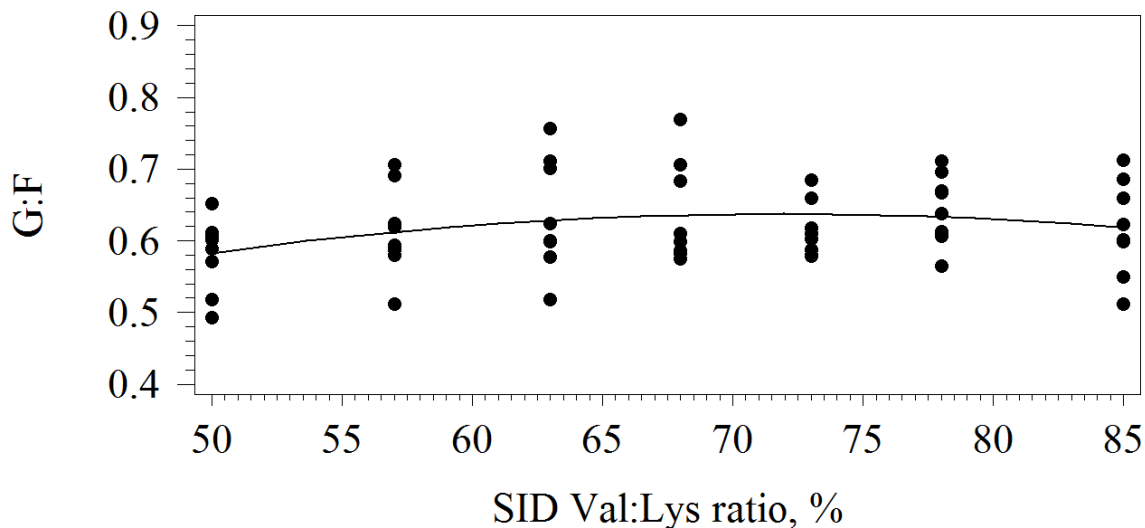
$$\text{ADFI} = -253.297 + 17.6999 \times (\text{SID Val:Lys}) - 0.1201 \times (\text{SID Val:Lys})^2$$



¹ A total of 280 nursery pigs (PIC 327 × 1050, initially 6.5 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then fed experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys ratio level to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Figure 2-5. Estimated standardized ileal digestible (SID) Val:Lys ratio requirement to maximize feed efficiency (G:F) for nursery pigs¹

G:F QP Maximum: 71.7% SID Val:Lys; 95% CI:(58, >85)
 99% Maximum: 64.6% SID Val:Lys
 $G:F = 0.010294 + 0.017526 \times (\text{SID Val:Lys}) - 0.000122 \times (\text{SID Val:Lys})^2$



¹ A total of 280 nursery pigs (PIC 327 × 1050, initially 6.5 kg BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then fed experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys level to maximize G:F, as well as SID Val:Lys level to achieve 99% of maximum G:F using the QP model. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Chapter 3 - Modeling the effects of standardized ileal digestible isoleucine to lysine ratio on growth performance of nursery pigs

Abstract

Two experiments evaluated the effects of increasing standardized ileal digestible (SID) Ile:Lys ratio on nursery pig growth performance. Treatments in both experiments contained 40, 44, 48, 52, 54, 58, or 63% SID Ile:Lys ratio. Diets were formulated using analyzed ingredient AA values and NRC (2012) SID coefficients and made by manufacturing the lowest and highest Ile:Lys ratio basal treatments and blending to create the intermediate treatments. There were 5 pigs per pen and 8 pens per treatment in both experiments. Data were analyzed separately for each experiment using heterogeneous variance where applicable and fitting 3 mixed models: quadratic polynomial (QP), broken-line linear (BLL), or broken-line quadratic (BLQ), selected for best-fit using Bayesian Information Criterion. In Exp. 1, 280 nursery pigs (PIC 327 × 1050, initially 6.7 ± 0.08 kg BW) were allotted to 1 of 7 SID Ile:Lys ratio treatments. Experimental diets were initiated 6-d post-weaning and fed for 12-d followed by a common diet from d 12 to 28. From d 0 to 12, increasing SID Ile:Lys ratio increased ADG (linear, $P < 0.005$) and ADFI (quadratic, $P < 0.017$) but G:F decreased (quadratic, $P < 0.043$). For ADG, the QP, BLL, and BLQ models reported maximum ADG at 64.7, 52.0, and 52.0% SID Ile:Lys ratio, respectively. For ADFI, the BLL breakpoint occurred at 50.6% and the QP predicted maximum ADFI at 56.2% SID Ile:Lys ratio. In Exp. 2, 280 nursery pigs (DNA 600 × 241, initially 6.0 ± 0.27 kg BW) were allotted to 1 of 7 treatments. Experimental diets were initiated 6-d post-weaning for 7 replications and 3-d post-weaning for 1

heavier replication and fed for 18-d followed by a common diet from d 18 to 32. During d 0 to 18, ADG and ADFI increased (quadratic, $P < 0.016$) with no evidence for difference in G:F as SID Ile:Lys ratio increased. For ADG, the QP and BLL had similar fit with breakpoints or maximums occurring at 58.3% and 51.8% SID Ile:Lys ratio, respectively. For ADFI, the BLQ breakpoint occurred at 52.0% SID Ile:Lys and the QP reported maximum ADFI at 57.2% SID Ile:Lys ratio. In conclusion, using the various statistical models presented, broken-line models reported maxima of 52.0% Ile:Lys ratio while quadratic models were as high as 64% of Lys to maximize ADG and ADFI of 6- to 11-kg nursery pigs.

Key words: amino acids, growth, isoleucine, nursery, pig

Introduction

In the nursery, swine diets can contain multiple synthetic AA including Lys, Met, Thr, Trp, Val, and Ile to meet the pig's requirements (Nørgaard and Fernández, 2009; Gloaguen et al. 2014) without a loss of performance. When formulating to the sixth-limiting AA, which is often Ile, NRC (2012) lists a 0.69% SID Ile requirement for pigs weighing 7- to 11- kg, suggesting a ratio of 51.1% SID Ile:Lys. Nørgaard et al. (2013), Htoo et al. (2014), and Soumeh et al. (2014) report similar estimates.

In previous research evaluating Ile requirements, the Lys requirement was unknown and could have been overestimated. This could cause the potential to underestimate the Ile requirement. Research evaluating requirements for Ile either uses high dietary levels of spray dried blood cells (SBDC), which contain low Ile and high Leu concentrations (NRC, 2012), or diets without SBDC where Ile was not low enough to determine a dose-response relationship. Excess Leu is problematic as it increases production of branched-chain keto-acid dehydrogenase, which metabolizes all BCAA,

causing an increased catabolism of Ile and raising the requirement (Langer et al., 2000; Wiltafsky et al., 2010; Morales et al., 2016).

Advancements in characterizing dose responses allow researchers to more accurately predict requirements. Gonçalves et al. (2016) detail modeling strategies to account for heteroskedasticity, a rather common phenomenon in animal agriculture (Cernicchiaro et al., 2013) characterized by unequal dispersion of residuals across treatments.

Thus, the objective of this study was to identify the Ile requirement of nursery pigs weighing approximately 6- to 11- kg via the use of recent statistical modeling methods.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment.

General

Similar protocols were used in both experiments. Each pen (1.52 × 1.52 m, Exp. 1; 1.52 × 1.22 m, Exp. 2) contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water. Both experiments were conducted at the Kansas State University Swine Teaching and Research Center.

Treatment diets were corn- and soybean meal-based containing 10% dried whey, 10% field peas, and 1.5% SDBC. Corn, soybean meal, field peas, and dried whey were analyzed for total AA content (excluding Trp; method 994.12; AOAC Int., 2012), Trp (method 13904:2005; ISO, 2005), and CP (method 990.03; AOAC Int., 2012) by Ajinomoto Heartland, Inc. (Chicago, IL) prior to formulation (Table 3-1). A new field pea batch and analysis prior to Exp. 2 called for a minor adjustment to Exp. 2 diets by

decreasing crystalline amino acids slightly. The SID digestibility coefficients were from NRC for all ingredients except field peas which were from Mathai (2015). Crystalline amino acids replaced corn in diets as treatment levels of Ile increased. With the exception of Lys and Ile, all other AA were formulated above their requirement estimates (NRC, 2012). Based on a previous study by Clark (2016), the Lys requirement for pigs of this weight range in these facilities was at least 1.45% SID Lys. Thus, experimental treatment diets were formulated to contain 1.28 and 1.24% SID Lys for Exp. 1 and 2, respectively, to ensure pigs were below their requirement and guarantee that Lys was second limiting. The 7 dietary treatments were 40, 44, 48, 52, 54, 58, and 63% SID Ile:Lys ratio. Basal diets were manufactured for the extreme treatments, and then blended at the feed mill to create the intermediate levels. A common phase was fed following experimental diets, and the diet was corn- and soybean meal- based containing no animal protein sources and formulated to 1.22% SID Lys. All diets were fed in meal form and prepared at the O.H. Kruse Feed Technology Innovation Center located in Manhattan, KS. Samples of experimental diets were submitted (Ward Laboratories, Kearney, NE) for analysis of DM (method 935.29; AOAC Int., 2012), CP (method 990.03; AOAC Int., 2012), crude fiber [method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY)], ash (method 942.05; AOAC Int., 2012), and ether extract [method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY). Samples were also analyzed for AA analysis using methods described above (Ajinomoto Heartland, Inc., Chicago, IL).

Experiment 1

A total of 280 nursery pigs (PIC 327 × 1050; 6.7 ± 0.08 kg BW) were used in a 26-d experiment with 8 pens per treatment and 5 pigs per pen. Pigs were weaned at

approximately 21 d of age and allotted to the nursery according to BW and gender. After 6 d in the nursery, pens were allotted to 1 of 7 dietary treatments by BW and location in a randomized complete block design. Treatment diets were fed for 12 d followed by a common diet for 14 d (Table 3-2). Pigs were weighed and feed disappearance was measured on d 0, 12, and 26.

Experiment 2

A total of 280 nursery pigs (DNA 600 × 241, initially 6.0 ± 0.27 kg BW) were used in a 32-d experiment. There were 8 pens per treatment and 5 pigs per pen. Pigs were weaned at approximately 20 d of age and allotted to pens according to BW, gender, and age. One replication was fed a common starter diet for 3 d due to heavier weaning BW and age, and then placed on experimental diets. The remaining 7 replications were fed a common starter diet for 6 d post-weaning before being placed on treatment diets. Day three for the first replication and d 6 for the remaining 7 replications were considered to be d 0, and pens were allotted to the dietary treatments by BW in a randomized complete block design. Treatments were fed for 18 d followed by a common diet for 14 d (Table 3-3). Pigs were weighed and feed disappearance was measured on d 0, 18, and 32.

Statistical Analysis

Statistical analyses for these experiments were performed using methods described by Gonçalves et al. (2016). Preliminary analyses steps included fitting a base mixed model to data for each experiment, recognizing pen as the experimental unit and a randomized complete block design. Base models were fitted using the GLIMMIX procedure of SAS (SAS Institute, Inc., 9.4, Cary, NC) with fixed effect of treatment and block as a random effect. The base model was also used to explore heterogeneity of residual variances during the experimental period when dietary treatments were fed

across treatments using Bayesian Information Criteria (BIC) to decide on best fitting approaches to account for heteroskedasticity. Models were evaluated separately for individual experiments, as variance components could not be estimated using data from only two experiments. After accounting for heterogeneous variance, base model linear and quadratic contrasts were evaluated, with coefficients for unequal spacing between dietary treatments. Results were considered significant at $P \leq 0.05$ and marginally significant at $P < 0.10$.

For Exp. 1, heterogeneous variance was applied for both ADG and G:F during the experimental period. Subsequently, evaluation of the base model linear and quadratic contrasts for feed efficiency resulted in quadratic effect in Exp. 1, with the lowest and the highest SID Ile:Lys ratios having the best G:F and numeric variation between the intermediate treatments. Due to the lack of biological explanation of this response, the dose response for G:F was not modeled. For Exp. 2, heterogeneous variance was applied for ADG and ADFI. The base models were then evaluated and due to a lack of dose response for feed efficiency the dose response curve was not modeled.

For both experiments competing continuous response dose-response models were fit to ADG and ADFI during the experimental periods (Exp. 1: d 0 to 12; Exp. 2: d 0 to 18) using PROC GLIMMIX and PROC NLMIXED according to procedures of Gonçalves et al. (2016). These procedures evaluated the functional forms of the relationship between ADG or ADFI and dietary treatments. The competing models evaluated were the quadratic polynomial (QP), broken-line linear (BLL), or broken-line quadratic (BLQ), following Gonçalves et al. (2016). The best-fitting dose-response model was decided using BIC, whereby a smaller BIC indicate a better fitting model; decreases of 2 points or more to indicate evidence for enhanced fit of the model with

lower BIC. These guidelines were based on the suggestions of Raftery (1996) and Gonçalves et al., (2016).

For the base model, treatment means and SEM were output. For each best fitting dose-response model, the individual pen means and modeled response curve were plotted. For the BLL and BLQ models breakpoints with the respective 95% CI were reported. For the QP model the maximum response and the CI for the maximum response was calculated by plotting the regression equation across the dose levels with 95% CI, then the maximum estimated response is projected on the y-axis using a horizontal line and points of intersection of this horizontal line with the CI boundaries on the predicted line are then projected onto the x-axis as CI estimators of the optimum dose level (Gonçalves et al., 2016).

Results

Amino acid analysis of ingredients resulted in corn generally being slightly higher in AA concentrations as compared to published values (NRC, 2012) with soybean meal being slightly lower. Analysis of AA for field peas in Exp. 1 and for Exp. 2 were similar to expected values.

Proximate analysis of experimental diets (Tables 3-4 and 3-5) generally matched formulated values. For a few AA analyses, the increase in Ile across treatments was less than expected but within analytical variation. Amino acid analyses of diets were reasonably consistent with diet formulation with Ile generally increasing across the treatments and other AA remaining relatively constant.

Experiment 1

From d 0 to 12 when experimental diets were fed, increasing SID Ile:Lys ratio increased ADG (linear, $P < 0.005$) and ADFI (quadratic, $P < 0.017$) (Table 3-6).

However, as SID Ile:Lys ratio increased, G:F decreased then increased (quadratic, $P < 0.043$) with the lowest and highest concentrations of 40% and 63% SID Ile:Lys ratio having the best G:F, resulting in a quadratic response. During the common phase (d 12 to 28), there was no evidence for differences in ADG, ADFI or G:F. During the overall period, ADG tended (linear, $P < 0.082$) to increase and ADFI increased (linear, $P < 0.011$) due to increasing SID Ile:Lys ratio in diets from d 0 to 12. Similarly, BW was increased (linear, $P < 0.006$) at the end of phase 1, but there was no evidence of treatment differences for final BW at the end of the common diet period.

For ADG (Figure 3-1) from d 0 to 12, the QP, BLL, and BLQ had similar competing fits (BIC = 558.3, 556.6, and 557.9, respectively). The BLL and BLQ reported similar breakpoints of 52.0% SID Ile:Lys ratio (95% CI: [51.96, 52.04%] and [51.97, 52.03%], respectively) with no further improvement in ADG found thereafter. The QP reported maximum ADG at 64.7% SID Ile:Lys ratio (95% CI: [51, >65%]) with 99% of maximum performance captured with 57.0% SID Ile:Lys ratio.

The estimated regression equation for the BLL model was:

$$\text{ADG, g} = 364.27 - 3.0272 \times (52.0 - \text{SID Ile:Lys}), \text{ when SID Ile:Lys} < 52.0\%;$$

$$\text{ADG, g} = 364.27, \text{ if SID Lys} \geq 52.0\%$$

where the SID Ile:Lys level is expressed as a percentage.

The estimated regression equation for the BLQ model was:

$$\text{ADG, g} = 365.68 - [5.6749 \times (52.0 - \text{SID Ile:Lys})] + [0.2344 \times (52.0 - \text{SID Ile:Lys})^2], \text{ when SID Ile:Lys} < 52.0\%;$$

$$\text{ADG, g} = 365.68, \text{ if SID Ile:Lys} \geq 52.0\%$$

The estimated regression equation for the QP model was:

$$\text{ADG, g} = 98.0474 + 8.4079 \times (\text{SID Ile:Lys}) - 0.0651 \times (\text{SID Ile:Lys})^2$$

For ADFI (Figure 3-2) from d 0 to 12, the BLL and QP resulted in competing fits (BIC = 603.8 and 604.4, respectively). The BLL breakpoint occurred at 50.6% SID Ile:Lys ratio (95% CI: [41.99, 59.15%]). The QP reported maximum ADFI at 56.2% SID Ile:Lys ratio (95% CI: [48, >65%]) with 99% of maximum intake captured at 51.6% SID Ile:Lys ratio.

The estimated regression equation for the BLL model was:

$$\text{ADFI, g} = 563.01 - 6.2844 \times (50.6 - \text{SID Ile:Lys}), \text{ when SID Ile:Lys} < 50.6\%;$$

$$\text{ADFI, g} = 563.01, \text{ if SID Ile:Lys} \geq 50.6\%$$

The estimated regression equation for the QP model was:

$$\text{ADFI, g} = -288.15 + 30.4124 \times (\text{SID Ile:Lys}) - 0.2705 \times (\text{SID Ile:Lys})^2$$

Experiment 2

From d 0 to 18 when experimental diets were fed, ADG and ADFI increased (quadratic, $P < 0.003$), but there was no evidence for differences in G:F as SID Ile:Lys ratio increased (Table 3-7). During the common period (d 18 to 32), there was no evidence for differences for ADG, but ADFI increased (linear, $P < 0.010$) and G:F decreased (linear, $P < 0.009$) for pigs previously fed diets with increasing SID Ile:Lys ratio. For the overall period (d 0 to 32), ADG and ADFI increased (quadratic, $P < 0.034$) with increasing SID Ile:Lys ratio with no differences in G:F. Finally, BW was increased (quadratic, $P < 0.032$) at the end of phase 1 and at the conclusion of the experiment with increasing SID Ile:Lys ratio.

For ADG (Figure 3-3) from d 0 to 18, the BLL and QP were competing best fit models (BIC = 541.8 and 543.3, respectively). The BLL breakpoint occurred at 51.8% SID Ile:Lys ratio (95% CI: [47.65, 55.93%]). The QP model resulted in maximum ADG

at 58.3% SID Ile:Lys ratio (95% CI: [49, >65%]) with 99% of maximum performance captured with 54.3% SID Ile:Lys ratio.

The estimated regression equation for the BLL model was:

$$\text{ADG, g} = 284.29 - 4.7304 \times (51.8 - \text{SID Ile:Lys}), \text{ when SID Ile:Lys} < 51.8\%;$$

$$\text{ADG, g} = 284.29, \text{ if SID Ile:Lys} \geq 51.8\%$$

The estimated regression equation for the QP model was:

$$\text{ADG, g} = -311.01 + 20.449 \times (\text{SID Ile:Lys}) - 0.1753 \times (\text{SID Ile:Lys})^2$$

For ADFI (Figure 3-4) from d 0 to 18 modeled with heterogeneous variance, the QP and BLQ resulted in similar competing fits (BIC = 591.0 and 591.7, respectively).

The BLQ breakpoint occurred at 52.0% SID Ile:Lys ratio (95% CI: [51.95, 52.05%]). The QP reported maximum ADFI at 57.2% SID Ile:Lys ratio (95% CI: [49, >65%]) with 99% of maximum intake captured at 53.5% SID Ile:Lys ratio.

The estimated regression equation for the BLQ model was:

$$\text{ADFI, g} = 419.44 - [6.1716 \times (52.0 - \text{SID Ile:Lys})] - [0.08475 \times (52.0 - \text{SID Ile:Lys})^2], \text{ when SID Ile:Lys} < 52.0\%;$$

$$\text{ADFI, g} = 419.44, \text{ if SID Ile:Lys} \geq 52.0\%$$

The estimated regression equation for the QP model was:

$$\text{ADFI, g} = -588.47 + 35.277 \times (\text{SID Ile:Lys}) - 0.3082 \times (\text{SID Ile:Lys})^2$$

Discussion

Numerous studies have been conducted to determine the Ile requirement of pigs of all weight ranges. Generally, researchers use one of two different approaches in diet formulation in diets, either with or without SDBC. Without SDBC it is difficult to obtain diet formulations to characterize the lower part of the dose response curve. The low Ile content of SDBC (NRC, 2012) has resulted in experiments using up to 7.5% SDBC.

Unfortunately, SDBC also contain high level of Leu. Low Ile content is desirable when formulating diets for an Ile titration. However, the Ile requirement may increase when other BCAAs are in excess, particularly due to an antagonistic effect with excess Leu. This mechanism occurs when elevated Leu increases levels of the enzyme complex branched-chain keto-acid dehydrogenase, which all BCAAs compete for in their respective degradation processes; thus catabolizing more Ile and increasing the requirement (Langer et al., 2000; Wiltafsky et al., 2010; Morales et al., 2016). Gietzen and Magrum (2001) also describe an anorexic response as a result of BCAA imbalance or deficiency. Thus, as feed intake is critical during the post-weaning period, it is important to understand the Ile requirement in relation to the level of SDBC in the diets. We used an alternative approach to diet formulation using 1.5% SDBC and diets that incorporated 10% field peas which allowed for further reduction of SID Ile:Lys but limiting SID Leu levels at no greater than 109% of Lys. The field peas were from a known batch of peas analyzed for total AA content and SID coefficients by Mathai (2015).

Nørgaard et al. (2013) evaluated the SID Ile:Lys ratio requirement for pigs weighing 8- to 18- kg and found that 52% SID Ile:Lys was sufficient in diets containing no blood products, agreeing with the results presented by broken line models in this experiment. Similarly, Soumeh et al. (2014) using SDBC-free diets found that 52% SID Ile:Lys ratio was the requirement for ADG and ADFI but that G:F was slightly lower at 48% SID Ile:Lys ratio for 8- to 15- kg pigs. Barea et al. (2009) evaluated individually-housed 11- to 23- kg pigs and determined that for diets without SDBC, no greater than 50% SID Ile:Lys was necessary.

Conversely, the Ile requirement seems to increase when SDBC are included in the diet due to antagonistic interactions previously described. Htoo et al. (2014) incorporated

SDBC ranging from 3.7 to 4.1% of the diet, depending on the phase. This strategy allowed diets to be below the estimated Ile requirement, but also mediate Leu levels at no greater than 130% of Lys. They observed a 51% and a 54% SID Ile:Lys ratio requirement for 10- to 22- kg and 24- to 39- kg pigs, respectively, determined using averages from both curvilinear and exponential regression models. Kerr et al. (2004) evaluated the apparent ileal digestible (AID) Ile:Lys requirement for 7- to 11- kg pigs using 7.5% dietary SDBC and found the requirement was 61% Ile of Lys. Similarly, Wiltafsky et al. (2009) using 7.5% dietary blood cells (1.61% SID dietary Leu) found a requirement as high as 59% SID Ile:Lys, but was only 54% in diets that included corn gluten rather than SDBC.

Our SID Ile estimates using quadratic models were much higher than levels in aforementioned literature, at 64% of Lys, while the broken-line models resulted in maxima very close to the NRC (2012) requirements. However, the quadratic models can be utilized to determine that 99% of maximum ADG or maximum ADFI can be achieved using 51-57% SID Ile:Lys ratio, which provides confidence in the 52% SID Ile:Lys ratio estimates from the broken-line models. It is important to consider estimating a response vs. estimating a requirement. The ability to apply subjective performance goals (i.e., 95 or 99% of maximum performance) allows nutritionists to determine the ideal AA level for a particular situation. These levels can vary depending on production goals and economics. Providing requirements for individual growth response criteria enables producers to, for instance, determine where 100% of maximum ADG can be captured, while still capturing 99% of another response. For example, in our Exp. 1, the QP predicted maximum feed intake at 56% SID Ile:Lys ratio, while reducing SID Ile:Lys to 52% would allow 99% of maximum feed intake, and this should also capture nearly

100% of ADG, as this is where the broken line models found their maxima. Making recommendations based on several criteria will ultimately provide the best economic return.

Factors that can affect requirement estimates for Ile include level of SDBC included in experimental diets and modeling techniques. However, our results are in general agreement with the majority of nursery studies that exclude or use lower levels of SDBC. In conclusion, these experiments demonstrate that the SID Ile requirement for 6- to 11- kg nursery pigs is approximately 52% of Lys for ADG and ADFI using broken line models and maximum response as high as 64% of Lys using quadratic models. These data validate that the Ile requirement for 6- to 11- kg pigs appears to be similar to NRC (2012) requirement estimates of 51.1% for the 7- to 11- kg nursery pig. However, as illustrated by the different competing models and range of 95% CI's the requirement should not be viewed as a point estimate but rather a dynamic range that has variability around the point estimate.

Literature Cited

- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2013. Comparative amino acid digestibility in US blood products fed to weanling pigs. *Anim. Feed Sci. Technol.* 181: 80-86. doi: 10.1016/j.anifeedsci.2013.03.002
- AOAC International. 2012. *Official Methods of Analysis of AOAC Int.* 19rd ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.
- Barea, R., L. Brossard, L. Floc'h, Y. Primot, and J. Van Milgen. 2009. The standardized ileal digestible isoleucine-to-lysine requirement ratio may be less than fifty percent in eleven-to twenty-three-kilogram piglets. *J. Anim. Sci.* 87:4022-4031. doi: 10.2527/jas.2009-1964
- Clark, A.B. Dose-responses to lysine, valine, and isoleucine and the effects of monosodium glutamate on nursery pigs. 2016. MS Thesis. Kansas State University, Manhattan.
- Cernicchiaro, N., D. G. Renter, S. Xiang, B. J. White, and N. M. Bello. 2013. Hierarchical Bayesian modeling of heterogeneous variances in average daily weight gain of commercial feedlot cattle. *J. Anim. Sci.* 91:2910–2919. doi: 10.2527/jas.2012-5543
- Gietzen, D. W., and L. J. Magrum. 2001. Molecular mechanisms in the brain involved in the anorexia of branched-chain amino acid deficiency. *J. Nutr.* 131:851S-855S. doi: 10.2527/jas.2009-1964
- Gloaguen, M., L. Floc'h, E. Corrent, Y. Primot, and J. Van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. *J. Anim. Sci.* 92:637-644. doi: 0.2527/jas.2013-6514

- Gonçalves, M. A. D., N. M. Bello, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, J. C. Woodworth, and R. D. Goodband. 2016. An update on modeling dose–response relationships: Accounting for correlated data structure and heterogeneous error variance in linear and nonlinear mixed models. *J. Anim. Sci.* 94:1940-1950. doi: 10.2527/jas2015-0106
- Htoo, J. K., C. L. Zhu, L. Huber, C. F. M. de Lange, A. D. Quant, B. J. Kerr, G. L. Cromwell, and M. D. Lindemann. 2014. Determining the optimal isoleucine:Lysine ratio for ten-to twenty-two-kilogram and twenty-four-to thirty-nine-kilogram pigs fed diets containing nonexcess levels of leucine. *J. Anim. Sci.* 92:3482-3490. doi: 10.2527/jas2013-6934
- ISO. 2005. Animal feeding stuffs – Determination of tryptophan content. ISO 13904:2005. 1st ed. Geneva, Switzerland.
- Kerr, B. J., M. T. Kidd, J. A. Cuaron, K. L. Bryant, T. M. Parr, C. V. Maxwell, and J. M. Campbell. 2004. Isoleucine requirements and ratios in starting (7 to 11 kg) pigs. *J. Anim. Sci.* 82:2333-2342.
- Langer, S., P. W. Scislowski, D. S. Brown, P. Dewey, and M. F. Fuller. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: Effects on plasma amino–and keto–acid concentrations and branched-chain keto-acid dehydrogenase activity. *Br. J. Nutr.* 83:49-58.
- Mathai, J. K. Effects of Fiber on the Optimum Threonine:Lysine Ratio in 25 to 50 kg Growing Gilts. 2015. MS Thesis. University of Illinois at Urbana-Champaign, Urbana.

- Morales, A., N. Arce, M. Cota, L. Buenabad, E. Avelar, J. K. Htoo, and M. Cervantes. 2016. Effect of dietary excess of branched-chain amino acids on performance and serum concentrations of amino acids in growing pigs. *J. Anim. Physiol. Anim. Nutr.* 100:39-45. doi: 10.1111/jpn.12327
- Nørgaard, J. V., and J. A. Fernández. 2009. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* 154:248-253. doi: 10.1016/j.anifeedsci.2009.08.010
- Nørgaard, J. V., A. Shrestha, U. Krogh, N. M. Sloth, K. Blaabjerg, H. D. Poulsen, P. Tybirk, and E. Corrent. 2013. Isoleucine requirement of pigs weighing 8 to 18 kg fed blood cell-free diets. *J. Anim. Sci.* 91:3759-3765. doi:10.2527/jas2012-5998
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Raftery, A. E. 1996. Approximate Bayes factors and accounting for model uncertainty in generalized linear regression models. *Biometrika* 83:251–266. doi:10.1093/biomet/83.2.251
- Soumeh, E. A., J. Van Milgen, N. Sloth, E. Corrent, H. D. Poulsen, and J. Nørgaard. 2014. The optimum ratio of standardized ileal digestible isoleucine to lysine for 8–15kg pigs. *Anim. Feed Sci. Technol.* 198: 158-165. doi: 10.1016/j.anifeedsci.2014.09.013
- Wiltafsky, M. K., M. W. Pfaffl, and F. X. Roth. 2010. The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. *Br. J. Nutr.* 103:964-976. doi: 10.1017/S0007114509992212

Wiltafsky, M., J. Bartelt, C. Relandeau, and F. X. Roth. 2009. Estimation of the optimum ratio of standardized ileal digestible isoleucine to lysine for eight-to twenty-five-kilogram pigs in diets containing spray-dried blood cells or corn gluten feed as a protein source. *J. Anim. Sci.* 87:2554-2564. doi: 10.2527/jas.2008-1320

Table 3-1. Chemical analysis of ingredients

Item, %	Corn ^{1,2}	Soybean meal ^{1,2}	Dried whey ^{1,2}	Field peas, Exp. 1 ³	Field peas, Exp. 2 ^{1,3}	Spray dried blood cells ⁴
Total AA						
Lys	0.29	2.88	0.79	1.54	1.59	8.88
Ile	0.31	2.09	0.65	0.89	0.91	0.28
Leu	1.10	3.51	1.07	1.52	1.59	12.62
Met	0.18	0.66	0.16	0.19	0.21	1.23
Thr	0.30	1.80	0.68	0.79	0.83	4.29
Trp	0.07	0.65	0.22	0.17	0.21	1.58
Val	0.40	2.12	0.59	0.99	0.99	8.35
His	0.24	1.17	0.18	0.50	0.54	3.17
Phe	0.43	2.35	0.37	1.03	1.07	7.25
Standardized ileal digestible AA, % (Calculated)						
Lys	0.21	2.56	0.77	1.40	1.44	8.67
Ile	0.25	1.86	0.62	0.78	0.79	0.25
Leu	0.95	3.09	1.05	1.35	1.41	12.3
Met	0.15	0.59	0.16	0.17	0.19	1.26
Thr	0.23	1.53	0.61	0.69	0.72	4.13
Trp	0.06	0.59	0.21	0.15	0.18	1.48
Val	0.32	1.84	0.56	0.86	0.86	8.14
His	0.20	1.05	0.17	0.46	0.50	6.07
Phe	0.36	2.07	0.34	0.92	0.96	7.08

¹ Analyzed at Ajinomoto Heartland, Inc. (Chicago, IL) for amino acid content.

² SID content calculated using SID coefficients from the NRC (NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC).

³ Exp. 1 peas used total AA content and SID coefficients from Mathai (2015). Exp. 2 peas use SID coefficients from Mathai (2015).

⁴ Spray dried blood cells use total values and coefficients from Almeida et al. (2013).

Table 3-2. Diet composition (Exp. 1, as-fed basis)¹

Item	Formulated SID ² Ile:Lys ratio, %		Common Phase
	40	63	
Ingredient, %			
Corn	57.68	57.59	63.77
Soybean meal, 48% CP	13.25	13.26	32.86
Dried whey	10.00	10.00	--
Field peas	10.00	10.00	--
Spray dried blood cells	1.50	1.50	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate, 22% P	1.80	1.80	1.10
Salt	0.30	0.30	0.35
L-Lys-HCl	0.63	0.63	0.30
DL-Met	0.33	0.33	0.12
L-Thr	0.32	0.32	0.12
L-Trp	0.10	0.10	--
L-Val	0.24	0.24	--
L-Ile	--	0.29	--
Glutamic acid	1.10	1.00	--
Glycine	1.10	1.00	--
Trace mineral premix ³	0.15	0.15	0.15
Vitamin premix ⁴	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00
Calculated analysis			
SID AA, %			
Lys	1.28	1.28	1.22
Ile:Lys	40	63	63
Leu:Lys	107	107	129
Met:Lys	42	42	33
Met and Cys:Lys	59	59	57
Thr:Lys	65	65	63
Trp:Lys	20.3	20.3	18.7
Val:Lys	71	71	69
Total Lys, %	1.38	1.38	1.37
ME, kcal/kg	3,228	3,236	3,272
NE, kcal/kg	2,427	2,436	2,407
SID Lys:ME, g/Mcal	3.96	3.95	3.73
SID Lys:NE, g/Mcal	5.36	5.34	5.16
CP, %	18.2	18.2	21.4
Ca, %	0.82	0.82	0.70
P, %	0.73	0.73	0.64
Available P, %	0.51	0.51	0.41

¹ Treatments 40% and 63% SID Ile:Lys were manufactured and blended at the feed mill to create the intermediate levels of 44, 48, 52, 54, and 58% SID Ile:Lys.

² Standardized ileal digestible.

³ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

⁴ Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 3-3. Diet composition (Exp. 2, as-fed basis)¹

Item	Formulated SID ² Ile:Lys ratio, %		Common Phase
	50	85	
Ingredient, %			
Corn	59.04	58.95	63.77
Soybean meal, 48% CP	11.95	11.96	32.86
Dried whey	10.00	10.00	--
Field peas	10.00	10.00	--
Spray dried blood cells	1.50	1.50	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate, 22% P	1.80	1.80	1.10
Salt	0.30	0.30	0.35
L-Lys-HCl	0.60	0.60	0.30
DL-Met	0.32	0.32	0.12
L-Thr	0.31	0.31	0.12
L-Trp	0.10	0.10	--
L-Val	0.23	0.23	--
L-Ile	--	0.28	--
Glutamic acid	1.10	1.00	--
Glycine	1.10	1.00	--
Trace mineral premix ³	0.15	0.15	0.15
Vitamin premix ⁴	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00
Calculated analysis			
SID AA, %			
Lys	1.24	1.24	1.22
Ile:Lys	40	63	63
Leu:Lys	109	109	129
Met:Lys	42	42	33
Met and Cys:Lys	60	60	57
Thr:Lys	66	66	63
Trp:Lys	21	21	18.7
Val:Lys	71	71	69
Total Lys, %	1.34	1.34	1.37
ME, kcal/kg	3,228	3,236	3,272
NE, kcal/kg	2,434	2,443	2,407
SID Lys:ME, g/Mcal	3.83	3.82	3.73
SID Lys:NE, g/Mcal	5.15	5.14	5.16
CP, %	18.6	18.6	21.4
Ca, %	0.81	0.81	0.70
P, %	0.73	0.73	0.64
Available P, %	0.51	0.51	0.41

¹ Treatments 40% and 63% SID Ile:Lys were manufactured and blended at the feed mill to create the intermediate levels of 44, 48, 52, 54, and 58% SID Ile:Lys.

² Standardized ileal digestible

³ Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron

sulfate; 73 g Zn from zinc sulphate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

⁴ Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

Table 3-4. Chemical analysis of diets (Exp. 1, as-fed basis)¹

Item	Formulated standardized ileal digestible (SID) Ile:Lys ratio, % ²						
	40	44	48	52	54	58	63
Proximate analysis, % ³							
DM	88.11	88.82	89.21	88.94	87.85	88.86	89.23
CP	18.0	18.7	18.6	18.7	18.8	18.8	19.0
Crude fiber	2.2	2.0	2.1	2.2	2.3	2.0	2.1
Ether extract	2.6	2.4	2.4	2.6	2.3	2.3	2.1
Ash	4.64	5.52	4.79	5.07	5.3	5.06	5.29
AA analysis, % ⁴							
Lys	1.28	1.34	1.40	1.42	1.39	1.45	1.41
Ile	0.59	0.67	0.74	0.74	0.79	0.86	0.93
Leu	1.46	1.50	1.52	1.52	1.53	1.51	1.56
Met	0.46	0.50	0.50	0.53	0.49	0.56	0.55
Met + Cys	0.75	0.79	0.79	0.74	0.76	0.84	0.83
Thr	0.93	0.92	0.89	0.97	0.87	0.91	0.95
Trp	0.24	0.26	0.27	0.28	0.26	0.28	0.29
Val	0.92	0.98	0.97	1.04	1.01	1.04	1.07
His	0.43	0.45	0.45	0.46	0.45	0.45	0.46
Phe	0.79	0.80	0.81	0.81	0.79	0.79	0.81

¹ Treatment diet samples were collected at the feed mill after manufacturing.

² Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) diets were blended at the feed mill to create the intermediate treatments.

³ Composite samples were submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

⁴ Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for AA analysis.

Table 3-5. Chemical analysis of diets (Exp. 2, as-fed basis)¹

Item:	Formulated standardized ileal digestible (SID) Ile:Lys ratio, % ²						
	40	44	48	52	54	58	63
Item, % ³							
DM	90.29	90.41	90.07	90.37	90.36	90.30	89.97
CP	18.1	18.4	18.6	18.2	18.3	18.7	18.7
Crude fiber	1.9	1.8	2.4	1.7	1.9	1.9	2.3
Ether extract	2.6	2.7	2.4	2.5	2.6	2.7	2.6
Ash	5.25	5.27	5.12	5.24	5.12	5.40	5.18
AA analysis, % ⁴							
Lys	1.33	1.34	1.34	1.36	1.36	1.35	1.33
Ile	0.60	0.60	0.65	0.69	0.75	0.75	0.82
Leu	1.47	1.43	1.45	1.47	1.48	1.47	1.48
Met	0.50	0.54	0.51	0.50	0.52	0.50	0.54
Met + Cys	0.75	0.77	0.73	0.75	0.76	0.77	0.82
Thr	0.86	0.84	0.92	0.87	0.90	0.91	0.98
Trp	0.27	0.27	0.27	0.25	0.27	0.26	0.27
Val	0.97	0.96	0.96	0.95	0.99	1.02	0.99
His	0.46	0.43	0.45	0.43	0.45	0.44	0.45
Phe	0.79	0.77	0.78	0.78	0.78	0.78	0.78

¹ Treatment diet samples were collected at the feed mill after manufacturing.

² Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) diets were blended at the feed mill to create the intermediate treatments.

³ Composite samples were submitted to Ward Laboratories (Kearney, NE) for analysis.

⁴ Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for AA analysis.

Table 3-6. Effects of increasing standardized ileal digestible (SID) Ile:Lys ratio on nursery pig growth performance, Exp. 1¹

Item	Formulated SID Ile:Lys ratio, % ²							SEM	Probability, <i>P</i> <	
	40	44	48	52	54	58	63		Linear	Quadratic
Treatment period (d 0 to 12)										
ADG, g	330	344	342	388	344	358	375	---	0.005	0.418
ADFI, g	495	524	546	601	522	574	555	16.6	0.002	0.017
G:F	0.669	0.657	0.628	0.648	0.658	0.625	0.676	---	0.904	0.043
Post-treatment period (d 12 to 26)										
ADG, g	554	555	557	553	573	576	545	14.5	0.779	0.325
ADFI, g	851	835	851	859	866	891	854	19.4	0.190	0.588
G:F	0.652	0.665	0.655	0.643	0.662	0.647	0.640	0.0102	0.166	0.429
Overall (d 0 to 26)										
ADG, g	450	458	458	477	467	475	467	11.0	0.082	0.270
ADFI, g	687	692	710	740	707	744	716	15.2	0.011	0.106
G:F	0.657	0.662	0.645	0.645	0.660	0.639	0.652	0.0092	0.337	0.504
BW, kg										
d 0	6.7	6.7	6.7	6.7	6.7	6.7	6.7	0.08	0.995	0.993
d 12	10.7	10.9	10.8	11.4	10.9	11.0	11.2	0.19	0.006	0.536
d 26	18.5	18.6	18.7	19.1	18.9	19.1	18.9	0.33	0.105	0.304

¹ A total of 280 nursery pigs (PIC 327 × 1050, initially 6.7 ± 0.08 kg BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 12 and a common diet was fed from d 12 to 26.

² Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) complete diets were blended upon manufacturing at the feed mill to create the 44, 48, 52, 54, and 58% SID Ile:Lys dietary treatments.

³ Heterogeneous variance resulted in SEM = 8.8 for 40, 48, 52, and 54% treatments and 14.0 for 44, 58, and 63% SID Ile:Lys ratio treatments.

⁴ Heterogeneous variance resulted in SEM = 0.0160 for 40, 48, 52, 58, and 63% treatments and 0.0096 for 54% SID Ile:Lys ratio treatment.

Table 3-7. Effects of increasing standardized ileal digestible (SID) Ile:Lys ratio on nursery pig growth performance, Exp. 2¹

Item	Formulated SID Ile:Lys ratio, % ²							SEM	Probability, <i>P</i> <	
	40	44	48	52	54	58	63		Linear	Quadratic
Treatment period (d 0 to 18)										
ADG, g	229	247	266	306	263	286	282	---	0.001	0.016
ADFI, g	331	370	393	453	395	421	410	---	0.001	0.003
G:F	0.690	0.671	0.679	0.677	0.666	0.682	0.687	0.0146	0.935	0.228
Post-treatment period (d 18 to 32)										
ADG, g	562	590	583	587	577	594	585	15.8	0.246	0.378
ADFI, g	852	906	896	940	902	928	925	25.8	0.010	0.154
G:F	0.661	0.653	0.652	0.625	0.642	0.640	0.633	0.0093	0.009	0.298
Overall (d 0 to 32)										
ADG, g	375	397	405	429	399	421	415	11.5	0.001	0.034
ADFI, g	559	605	613	666	615	643	635	18.6	0.001	0.010
G:F	0.670	0.658	0.661	0.645	0.651	0.655	0.653	0.0090	0.107	0.209
BW, kg										
d 0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	0.27	0.824	0.920
d 18	10.1	10.5	10.8	11.5	10.8	11.2	11.1	0.41	0.001	0.010
d 32	18.0	18.7	19.0	19.7	18.9	19.5	19.3	0.59	0.001	0.032

¹ A total of 280 nursery pigs (DNA Genetics Line 600 × Line 241, initially 6.0 ± 0.27 kg BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 18 and a common diet was fed from d 18 to 32.

² Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) complete diets were blended upon manufacturing at the feed mill to create the 44, 48, 52, 54, and 58% SID Ile:Lys dietary treatments.

³ Heterogeneous variance resulted in SEM = 11.5 for 40, 44, 52, 54, 58, and 63% treatments and 3.5 for 48% SID Ile:Lys ratio treatment.

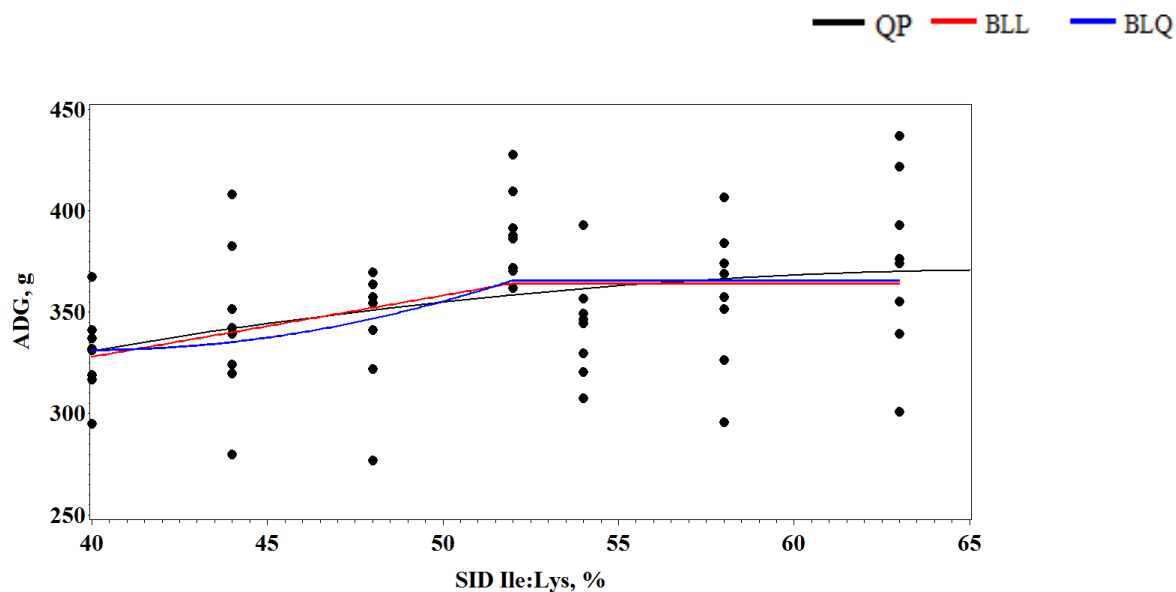
⁴ Heterogeneous variance resulted in SEM = 17.3 for 40, 44, 52, 54, 58, and 63% treatments and 7.9 for 48% SID Ile:Lys ratio treatment.

Figure 3-1. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 1¹

QP BIC= 558.3 (95% CI: [51, >65%])
 Max 64.7% SID Ile:Lys;
 99% of Max 57.0 % SID Ile:Lys
 $ADG = 98.05 + 8.408 \times (SID \text{ Ile:Lys}) - 0.0651 \times (SID \text{ Ile:Lys})^2$

BLL BIC= 556.6
 Breakpoint 52.0% SID Ile:Lys
 95% CI: [51.96, 52.04]
 $ADG = 364.27 - 3.027 \times (52.0 - SID \text{ Ile:Lys})$, when SID Ile:Lys < 52.0%;
 $ADG = 364.27$, if SID Ile:Lys \geq 52.0%

BLQ BIC= 557.9
 Breakpoint 52.0% SID Ile:Lys
 95% CI:[51.97, 52.03]
 $ADG = 365.68 - [5.675 \times (52.0 - SID \text{ Ile:Lys})] + [0.2344 \times (52.0 - SID \text{ Ile:Lys})^2]$,
 when SID Ile:Lys < 52.0%;
 $ADG = 365.68$, if SID Ile:Lys \geq 52.0%



¹ A total of 280 nursery pigs (PIC 327 \times 1050, initially 6.7 ± 0.08 kg BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 12 and a common diet was fed from d 12 to 26. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Figure 3-2. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 1¹

QP BIC= 604.4

Max 56.2% SID Ile:Lys

95% CI:[48, >65%]

99% of Max 51.6% SID Ile:Lys

$ADFI = -288.15 + 30.4124 \times (SID \text{ Ile:Lys}) - 0.2705 \times (SID \text{ Ile:Lys})^2$

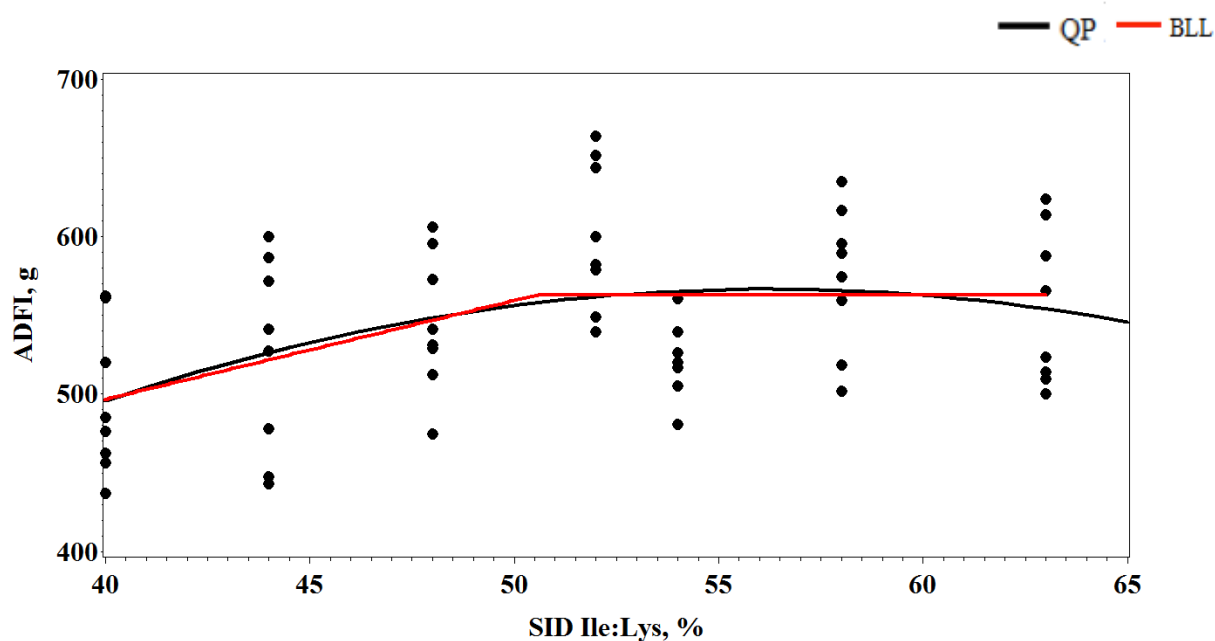
BLL BIC= 603.8

Breakpoint 50.6% SID Ile:Lys

95% CI:[41.99, 59.15]

$ADFI = 563.01 - 6.2844 \times (50.6 - SID \text{ Ile:Lys})$, when SID Ile:Lys < 50.6%;

$ADFI = 563.01$, if SID Ile:Lys \geq 50.6%



¹ A total of 280 nursery pigs (PIC 327 \times 1050, initially 6.7 ± 0.08 kg BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 12 and a common diet was fed from d 12 to 26. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Figure 3-3. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 2¹

QP BIC= 543.3

Max 58.3% SID Ile:Lys

95% CI:[49, >65%]

99% of Max 54.3% SID Ile:Lys

$$\text{ADG} = -311.0 + 20.449 \times (\text{SID Ile:Lys}) - 0.1753 \times (\text{SID Ile:Lys})^2$$

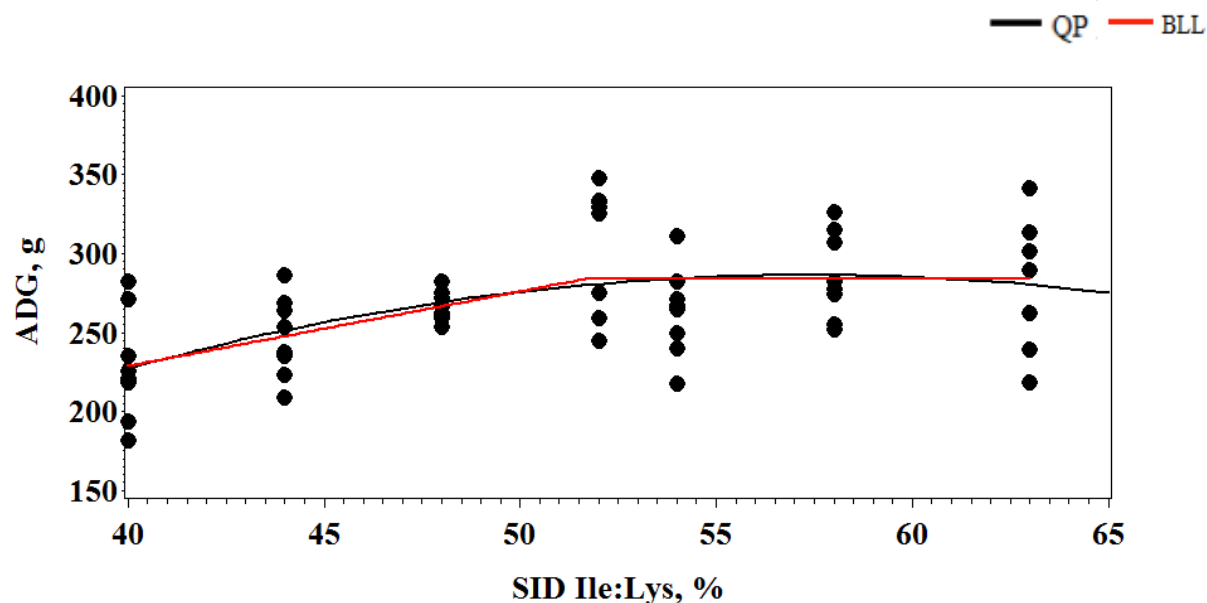
BLL BIC= 541.8

Breakpoint 51.8% SID Ile:Lys

95% CI:[47.65, 55.93]

ADG = 284.29 - 4.73 × (51.8 - SID Ile:Lys), when SID Ile:Lys < 51.8%;

ADG = 284.29, if SID Ile:Lys ≥ 51.8%



¹ A total of 280 nursery pigs (DNA 600 × 241, initially 6.0 ± 0.27 kg BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 18 and a common diet was fed from d 18 to 32. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

Figure 3-4. Estimated standardized ileal digestible (SID) Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 2¹

QP BIC= 591.0

Max 57.2% SID Ile:Lys

95% CI:[49, >65%]

99% of Max 53.5% SID Ile:Lys

$$\text{ADFI} = -588.47 + 35.277 \times (\text{SID Ile:Lys}) - 0.3082 \times (\text{SID Ile:Lys})^2$$

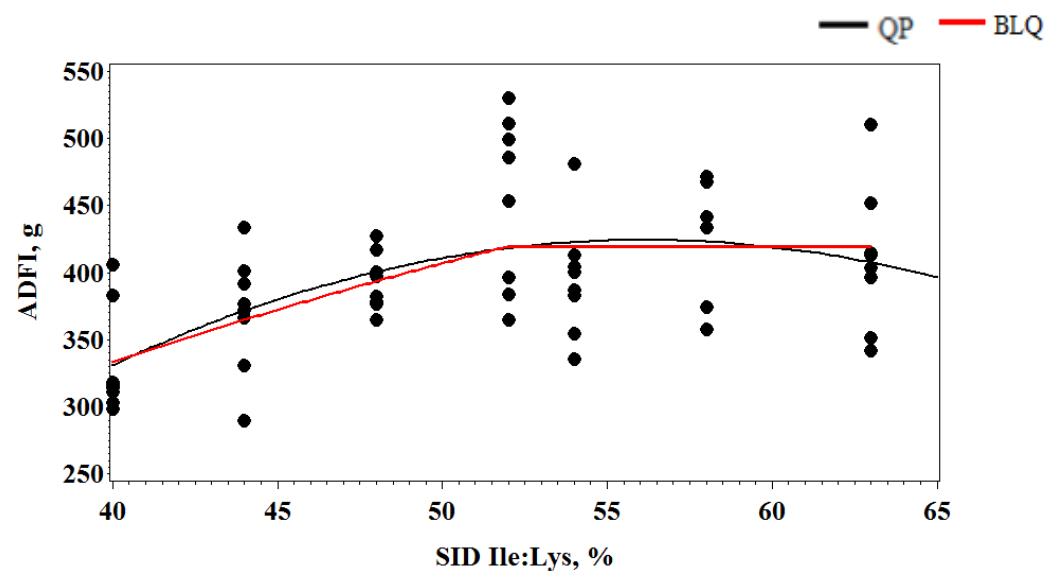
BLQ BIC= 591.7

Breakpoint 52.0% SID Ile:Lys

95% CI: [51.95, 52.05]

$$\text{ADFI} = 419.44 - [6.172 \times (52.0 - \text{SID Ile:Lys})] - [0.0848 \times (52.0 - \text{SID Ile:Lys})^2], \text{ when SID Ile:Lys} < 52.0\%;$$

$$\text{ADFI} = 419.44, \text{ if SID Ile:Lys} \geq 52.0\%$$



¹ A total of 280 nursery pigs (DNA 600 × 241, initially 6.0 ± 0.27 kg BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 18 and a common diet was fed from d 18 to 32. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.