Effects of phytase and mycotoxin control strategies in nursery pig diets and determining finishing pig lysine requirements

by

Larissa Lynn Becker

B.S., Iowa State University, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

Approved by:

Co-Major Professor
Dr. Joel DeRouchey

Approved by:

Co-Major Professor
Dr. Jordan Gebhardt

Copyright

Abstract

This thesis includes three chapters addressing very practical questions facing swine nutritionists including developing a phytase release curve to quantify the release of plant-based phosphorous in swine diets, determination of the lysine requirements in finishing diets, and control strategies for diets with high levels of deoxynivalenol (DON). One experiment using a total of 360 nursery pigs was used to determine the effect of increasing phytase on nursery pig growth performance and bone ash characteristics. Two experiments using a total of 4,223 pigs were used to determine the optimal dietary standardized ileal digestible (SID) lysine (Lys) in duroc-based sired finishing pigs. Additionally, one experiment using 4,318 pigs was conducted to evaluate dietary mycotoxin control strategies on nursery pig performance and blood measures. Experiment 1 determined the available phosphorus (aP) release of GraINzyme phytase in nursery pigs. Increasing phytase from 150 to 1,500 FTU/kg in phosphorus deficient diets improved nursery pig growth performance and bone ash characteristics. Using growth performance, bone ash weight, percentage bone ash, and formulated phytase concentrations, equations were developed to predict aP release up to 1,500 FTU/kg of GraINzyme phytase for 10- to 20- kg pigs. Experiments 2 and 3 were conducted to determine the optimal dietary SID Lys in finishing pigs. Experiment 2 determined that increasing SID Lys improved growth performance and final BW. Furthermore, feed cost, feed cost/kg of gain, revenue, and income over feed cost (IOFC) increased with increasing SID Lys. In experiment 3, increasing SID Lys increased growth performance, overall market weight, and HCW. Additionally, feed cost, revenue, and IOFC increased with increasing SID Lys. Experiment 4 determined that pigs fed diets contaminated with high concentrations of DON had decreased growth performance compared to pigs fed diets

contaminated with low DON concentrations. Furthermore, when feed additives such as sodium metabisulfite (SMB), Technology1, or Technology1+ were included in high DON diets, SMB supplementation led to growth performance that exceeded pigs fed the low DON diets. In summary, these experiments provide data on aP release of GraINzyme phytase, SID Lys requirements in finishing pigs, and mycotoxin control strategies in swine diets.

Table of Contents

List of Figures

List of Tables

Acknowledgements

This thesis was made possible by the generous support and encouragement by many people. I'd first like to thank my major professors, Dr. Joel DeRouchey and Dr. Jordan Gebhardt for their guidance and mentorship that has continued to shape my passion for the swine industry. Thank you to Dr. Tokach, Goodband, and Woodworth, your knowledge and encouragement made my graduate school experience enjoyable. You all continue to push and challenge me to become the best version of myself in all areas of life. It is humbling to be trained and educated by some of the best swine nutritionists in the industry.

I am thankful for the past and present graduate students of the applied swine nutrition team. You have become more than colleagues. Thank you for creating a fun, yet hardworking atmosphere and developing the team tradition. Graduate school would not be as much fun without all of your support inside and outside of the barns.

Lastly, to my family. Mom and dad, thank you for sharing your passion for the swine industry with me at such a young age. You have instilled in me a hard work ethic, determination, and a strong faith that helps me accomplish anything I put my mind to. Leanna, Levi, Luke, and Lance, thank you for shaping me into the individual that I am today and for always supporting me as I chase my dreams. Mahoney, Nevaeh, Amaya, and Jada, thank you all for the encouragement and letting me share my passion of pigs with all of you.

Chapter 1 - Determining the phosphorus release of GraINzyme phytase in diets for nursery pigs

Abstract

The objective of this study was to determine the available P (aP) release curve for a new phytase source, GraINzyme Phytase (Agrivida Inc., Woburn, MA), which is expressed in corn containing an engineered *Escherichia coli* phytase called Phy02. Plant-expressed phytases are created by inserting phytase-encoding genes into plants resulting in their ability to produce seeds with increased concentrations of phytase. A total of 360 pigs (Line 200 × 400, DNA, Columbus, NE, initially 9.9 ± 0.19 kg) were used in a 21-d growth study. Pigs were weaned at approximately 21-d of age, randomly allotted to pens based on initial body weight (BW) and fed common starter diets. From d 18 to 21 post-weaning, all pigs were fed a diet containing 0.11% aP. On d 21 post-weaning, considered d 0 of the study, pens were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 5 pigs per pen and 9 pens per treatment. Dietary treatments were formulated to include increasing aP derived from either an inorganic P source (0.11, 0.19, or 0.27% from monocalcium P) or increasing phytase (150, 250, 500, 1,000, or 1,500 FTU/kg). Diets were corn-soybean meal-based and contained 1.24% standardized ileal digestible (SID) Lys. On d 21 of the trial, 1 pig per pen (weighing closest to the mean pen BW) was euthanized and the right fibula was collected to determine bone ash using the non-defatted processing method. Overall (d 0 to 21), pigs fed increasing aP from inorganic P or phytase had increased (linear, $P < 0.002$) ADG, ADFI, and G:F (quadratic, $P < 0.05$). Bone ash weight (g) and percentage bone ash increased (linear, $P < 0.001$) with increasing inorganic P or added phytase. Based on the composition of the diets used in this study, the release equations developed for GraINzyme for ADG, G:F, bone ash weight, and percentage bone ash are: aP =

(0.255 × FTU) ÷ (1299.969 + FTU), aP = (0.233 × FTU) ÷ (1236.428 + FTU), aP = (45999.949 × FTU) ÷ (462529200 + FTU), and aP = (0.272 × FTU) ÷ (2576.581 + FTU), respectively.

Key Words: bone ash, nursery pigs, phosphorus, phytase

Introduction

Most swine diets are formulated using ingredients which contain phytate-bound-phosphorus. Phytic acid or phytate is a six-fold dihydrogen phosphate ester of inositol that is the major storage form (60 to 82%) of P in grains (Ravindran et al., 1994). Monogastric species do not naturally synthesize the enzyme phytase to cleave the phosphates from the phytic acid for absorption (Selle and Ravindran, 2007). Therefore, exogenous phytase can be added to swine diets to make P more available for growth and bone development (Cromwell et al., 1993; Bento et al., 2012; Rutherford et al., 2014). The added phytase allows for reduced dietary additions of inorganic P which results in reduced P excretion and lower feed cost (Selle and Ravindran, 2007; Li et al., 2013).

Phytase was first commercialized in 1991 and is known to be one of the most significant discoveries in animal nutrition (Cromwell, 2009; Li et al., 2013). While some phytase products have already undergone evaluation to determine their unique P release curve, other newer products have to be thoroughly tested as they become available (Jones et al., 2010; Goncalves et al., 2016; Gourley et al., 2018; Wensley et al., 2020). GraINzyme Phytase (Agrivida, Woburn, MA) is expressed in corn that contains an engineered *Escherichia coli* phytase called Phy02 (Ligon, 2016). Previous research conducted by Nyannor et al. (2007) using a corn-expressed phytase (660 FTU per g) in young pig diets observed a linear increase in ADG and G:F with increasing phytase added to a diet deficient in P.

GraINzyme corn-expressed phytase improves ADG, feed efficiency, bone mineralization, and bone strength when fed to young pigs (Lee et al., 2017; Blavi et al., 2019; Munoz Alfonso et al., 2018; Broomhead et al., 2019). Previous research evaluated the effects of corn-expressed phytase on growth performance, bone characteristics, and digestibility, with other microbial phytase sources. However, these studies have only evaluated the effects of increasing dosage of GraINzyme in P deficient diets without comparison to an inorganic P source to develop a P release curve. Therefore, the objective of this study was to evaluate the effects of GraINzyme phytase on nursery pig growth performance and bone ash to develop an aP release curve.

Materials and Methods

The Kansas State University Animal Care and Use Committee approved the protocol (4035) used in this experiment. Corn, soybean meal, limestone and monocalcium phosphate were analyzed for Ca and P (AOAC 985.01 and 985.01, respectively AOAC 2006) to determine nutrient loading values used for formulation prior to the manufacturing of diets (Table 1). Phytase was acquired for the experiment and analyzed (Agrivida Inc., Woburn, MA) using an adjusted extraction procedure that employed an optimized buffer and extraction process specifically for GraINzyme phytase (Li and Raab, 2016). Phytase activity was 9,738,000 FTU/kg. All diets were formulated to contain a Ca:P ratio of 1.10:1 with no allowance for release of Ca by phytase. The diets were corn-soybean meal-based and calculated to contain approximately 0.26% phytate P (NRC, 2012). Diets were formulated to 1.24% SID Lys with other amino acids set to meet or exceed NRC (2012) requirement estimates.

Diet Manufacturing

GraINzyme Phytase was ground in a roller mill (California Pellet Mills, Waterloo IA) to a similar particle size (approximately 600 microns) as the other corn in the diet. At the time of

manufacturing, 5 identical 1,814 kg batches of basal diet were produced and packaged in 22.7 kg bags at Hubbard Feeds in Beloit, KS (Table 2). For each experimental diet, a subset of bags from each batch of basal diet was added to the mixer along with treatment specific ingredients including sand, limestone, monocalcium P, and GraINzyme phytase to achieve the final experimental diets (Table 3). These diets were mixed at the O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Complete diets samples were taken during the bagging of experimental diets with a sub-sample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. After homogenization, samples were stored at -20ºC until they were submitted for phytase and nutrient analysis.

Animals and Housing

The experiment was conducted at the Kansas State University Segregated Early Wean Facility in Manhattan, KS. The nursery barns were environmentally controlled, and each pen contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water.

A total of 360 barrows (Line 200 × 400, DNA, Columbus, NE; initially 9.9 ± 0.19 kg) were used in a 21-d growth trial. Pigs were weaned at approximately 21-d of age, randomly allotted to pens based on initial BW, and fed common phase 1 and 2 diets after placement for 18-d. From d 18 to 21 post-weaning, all pigs were fed a diet containing 0.11% aP. On d 21 post-weaning, considered d 0 of the study, pens of pigs were blocked by weight and randomly allotted to 1 of 8 dietary treatments with 5 pigs per pen and 9 pens (weight blocks) per treatment. Treatments consisted of 3 diets with increasing P from monocalcium P formulated to provide 0.11, 0.19, or 0.27% aP, or 5 diets with 150, 250, 500, 1,000, or 1,500 FTU/kg phytase added to the diet containing 0.11% aP.

During the experiment, pigs and feeders were individually weighed every 7 d to determine ADG, ADFI, and G:F. At the conclusion of the study, one pig in each pen closest to the mean BW was euthanized via penetrating captive bolt and the right fibula was collected to determine bone ash weight and percentage bone ash (72 barrows, 9 observations per treatment). After collection, bones were individually placed in plastic bags with permanent identification and stored at -20°C until processing. On the day of processing, bones were autoclaved for 1 hour at 121°C. After cooling, any leftover extraneous soft tissue including cartilage caps was cleaned from the fibulas. Fibulas (one from each pig) were dried at 105°C for 7 d in a drying oven, reweighed and then ashed at 600°C for 24 h to determine total ash weight and percentage ash relative to dried bone weight (Wensley et al., 2020).

Chemical Analysis

Three samples per dietary treatment from the pooled feed samples were sent to the KSU Soils Lab in Manhattan, KS for duplicate analysis of Ca (AOAC 985.01, 2006) and P (AOAC 985.01, 2006). Additionally, five samples of each diet were submitted for phytase analysis (Agrivida Inc., Woburn, MA) using an extraction procedure that employed an optimized buffer, (pH 10.8) as described by Li and Raab (2016) which is different from the AOAC method (Gizzi et al., 2008).

Statistical Analysis

Studentized residuals were evaluated for pen means or individual bone ash measurements to ensure data met the assumption of normal distribution. Data were analyzed as a randomized complete block design with pen as the experimental unit. An initial base model was evaluated using the MIXED procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Treatment was considered a fixed effect and weight block a random effect. Linear and quadratic

contrasts were evaluated within increasing inorganic P or phytase treatments. Contrast coefficients were adjusted to account for unequal spacing in phytase doses using the IML procedure. For pens of pigs fed the inorganic P diets, the marginal intake of aP per day was calculated for each pen using the equation: aP intake/day = [dietary aP% - 0.11% (aP in the basal diet)] × ADFI. A standard curve was then developed for each response criteria using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criteria. This value was then converted to a marginal aP% using the pen ADFI.

A mixed model ANOVA with weight block as a random effect was performed to evaluate aP release as a function of phytase dosage, assuming an intercept of no aP release for the control diet without phytase. All release values were calculated using formulated P and phytase levels. The GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC) was used for the analysis.

Release values were used in a non-linear regression to fit a model predicting aP release curves dependent on phytase dosage as a continuous variable using the individual pen data using the following functional form:

$$Available\ P\ release, \% = \frac{a \times FTU}{b + FTU}$$

Where coefficient a is a horizontal asymptote representing the maximum release of aP for the given response, and coefficient -b represents the vertical asymptote. Separate aP release curves were developed using aP release derived from ADG, G:F, percentage bone ash, and bone ash weight. Model parameters were estimated using the nls function from the R Stats Package [Version 4.0.2 (2020-06-22); R Core Team, 2020] using the RStudio environment (Version

1.3.1093; RStudio Team, 2020). Results were considered significant with *P*-values ≤ 0.05 and considered marginally significant with *P*-values > 0.05 and ≤ 0.10.

Results

Analysis of manufactured diets resulted in similar Ca and P values to those expected from diet formulation (Table 3). Phytase analysis of complete diets showed a stepwise increase in phytase activity as expected and were similar to formulated values for each phytase containing diet.

From d 0 to 21, pigs fed increasing aP from inorganic P had increased (linear, $P \leq 0.002$) ADG, ADFI, G:F, and final BW (Table 4). In addition, pigs fed diets with increasing phytase had improved (linear, $P < 0.001$) growth performance across all response variables. There was a quadratic ($P = 0.049$) increase in G:F when the level of dietary phytase increased. For bone characteristics, bone ash weight and percentage bone ash increased (linear, $P \leq 0.003$) for pigs fed either increasing inorganic P or phytase in the diet.

The percentage aP released from GraINzyme varied depending on the response criteria measured (Table 5). As the amount of phytase in the diet increased, the calculated aP release increased (linear, $P \leq 0.008$) for ADG, bone ash weight, and percentage bone ash. For G:F, there was a quadratic ($P < 0.045$) increase in aP release as the amount of phytase in the diet increased. At the low levels of phytase inclusion, release of aP was not evident based on negative release values for bone ash weight and bone ash percentage. However, when using the modelling approach there is evidence of a small amount of aP release at the low phytase levels for bone mineralization measures. The release equations generated from this experiment for GraINzyme for ADG (Figure 1), G:F (Figure 2), bone ash weight (Figure 3), and percentage bone ash (Figure 4) are: $aP = (0.255 \times FTU) \div (1299.969 + FTU)$, $aP = (0.233 \times FTU) \div (1236.428 + FTU)$, aP =

(45999.949 × FTU) ÷ (462529200 + FTU), and aP = (0.272 × FTU) ÷ (2576.581 + FTU), respectively. When viewed together, the aP release for ADG and G:F is greater at any given FTU/kg compared to bone ash percentage (Figure 5). Release of aP as indicated by bone ash weight was less than ADG and G:F at lower FTU/kg levels, but increased as FTU/kg level increased relative to growth performance measures.

Discussion

Swine and poultry produce low amounts of endogenous phytase and as a result, are unable to cleave much of the phytate-bound P in cereal grains (Humer et al., 2015). Consequently, microbial phytase is commonly added to swine diets to make phytate-bound P available. Previous research has observed that there are several beneficial effects when microbial phytase is added to P deficient diets (Dersjant-Li et al., 2015) including improved digestibility and growth performance as well as less P in swine waste which benefits the environment (Selle and Ravindran, 2008).

The first sources of phytase were fungal-derived and commercialized in 1991 (Dersjant-Li et al., 2015). Later, Rodriguez et al. (1999) observed that *Escherichia coli* phytases were more effective than fungal phytases leading to the development of new phytase sources. Phytase sources can be categorized as 3- and 6-phytases, depending on the carbon site of hydrolysis (Augspurger et al., 2003). The GraINzyme phytase used in this study, is expressed in corn, *E. coli*-derived, and classified as a 6-phytase.

Microbial phytase may be expressed in plants such as soybeans and canola seed. Previous research observed that phytases expressed in soybeans (Denbow et al., 1998) and canola seeds (Zhang et al., 2000) had similar performance compared to microbial phytases. However, because of the insufficient thermo-tolerance of these phytases, they were unable to survive the

postharvest heating that occurs during the toasting of defatted meals (Haefner et al., 2005). Conversely, corn-expressed phytase, like GraINzyme, is likely to maintain efficacy after harvest because of the cooler temperature used in drying (if any) corn compared that used in the toasting of oilseed meal (Nyannor et al., 2007).

GraINzyme contains an engineered *E. coli* phytase called Phy02 (Ligon, 2016). Phy02 phytase is expressed in the corn *(Zea mays)* kernel and then ground into a course meal to improve the digestibility of phytate-bound P. Wang and Kim (2020) evaluated the growth performance and bone mineralization of poultry fed 500 to 4,500 FTU/kg of GraINzyme. Broiler chicks fed increasing levels of phytase had a linear ($P < 0.001$) increase in body weight gain, feed intake, and percentage bone ash.

GraINzyme has been demonstrated to improve ADG, feed efficiency, bone mineralization, and bone strength when young pigs were fed reduced P and Ca diets (Lee et al., 2017; Blavi et al., 2019; Munoz Alfonso et al., 2018). Broomhead et al. (2019) evaluated 500 to 4,000 FTU/kg from GraINzyme in a reduced P nursery pig diet. As phytase dose increased, the apparent total tract digestibility (ATTD) of P, ADG, ADFI, and percentage bone ash increased in a quadratic ($P \leq 0.003$) fashion. Blavi et al. (2019) evaluated the growth performance, bone ash measurements, and digestibility of Ca and P in young pigs fed 250 to 1,500 FTU/kg GraINzyme in a Ca and P deficient diet. Like Broomhead et al. (2019), as phytase dose increased, the growth performance, percentage bone ash, and ATTD of Ca and P improved in a quadratic ($P \leq 0.054$) fashion. Similarly, in the present study increasing phytase inclusion from 150 to 1,500 FTU/kg resulted in linear ($P < 0.001$) improvements in ADG, ADFI, G:F, and percentage bone ash. Similar to Broomhead et al. (2019), while there was a strong linear ($P < 0.001$) effect on percentage bone ash, there was a slight decrease in the pigs fed 1,000 FTU/kg of GraINzyme.

Because of the intrinsic differences between phytase sources, it is important to evaluate their unique aP release when new or enhanced phytase sources enter the marketplace. A common approach used for comparing phytase sources is to compare the phytase activity needed to reach a particular aP release value allowing products to be compared on the same level of activity. Wensley et al. (2020) observed that Smizyme TS G5 2,500 was able to achieve a 0.12 aP at 500 FTU/kg using percentage bone ash. Gourley et al. (2018) observed that Natuphos E 5,000 G was able to achieve a 0.11 aP release based on percentage bone ash with 500 FTU/kg. In our study, GraINzyme, using percentage bone ash as the criteria, would need to be added to diets at a level greater than 1,500 FTU/kg in order to achieve a similar aP release of 0.12. Using a modelling approach, the aP release increases as FTU/kg increases for all response criteria as would be expected. This is consistent with previous works with low levels of release at the lower levels and increasing release as FTU/kg increases. Depending on the response criteria used, it appears that GraINzyme may need to be included at a greater number of FTU/kg in order to obtain a similar response observed with other commercial phytase sources. One speculation is that the specific extraction method for GraINzyme may lead to higher FTU values. Based on the FTU reported by Broomhead et al. (2019), the values obtained from the Li and Raab (2016) extraction method are on average 64% greater than the values from the AOAC method. This might suggest that if the AOAC method was used, it would have likely provided lower FTU values, and that fewer FTUs are required for the same aP release.

In conclusion, using the diet formulations in the present study, an aP release curve can be developed and used for GraINzyme phytase as a means to improve aP digestibility in nursery diets when included at concentrations between 150 and 1,500 FTU/kg. The magnitude of aP release at different FTU inclusion rates depends on the response criteria measured.

References

AOAC. 2006. Official methods of analysis AOAC international. 18th ed. Association of Official Analytical Chemists, Arlington, VA.

Augspurger, N.R., D.M. Webel, X.G. Lei, and D.H. Baker. 2003. Efficacy of an E. coli phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. J. Anim. Sci. 81:474-483. doi:10.2527/203.812474x

Bento, M. H. L., C. Pedersen, P. W. Pleumstead, L. Salmon, C. M. Nyachoti, and P. Bikker. 2012. Dose response of a new phytase on dry matter, calcium, and phosphorus digestibility in weaned piglets. J. Anim. Sci. 90(Suppl. 4):245-247. doi:10.2527/jas.53988

Blavi, L., C. J, Munoz, J. N. Broomhead, and H. H. Stein. 2019. Effects of a novel corn-expressed *E. coli* phytase on digestibility of calcium and phosphorus, growth performance, and bone ash in young growing pigs. J. Anim. Sci. 97:3390-3398. doi:10.1093/jas/skz190

Broomhead, J. N., P. A. Lessard, R. M. Raab, and M. B. Lanahan. 2019. Effects of feeding corn-expressed phytase on the live performance, bone characteristics, and phosphorus digestibility of nursery pigs. J. Anim. Sci. 97:1254-1261. doi:10.1093/jas/sky479

Cromwell, G. L. 2009. ASAS Centennial Paper: Landmark discoveries in swine nutrition in the past century. J. Anim. Sci. 87:778-792. doi:10.2527/jas.2008-1463

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71:1831-1840. doi:10.2527/1993.7171831

Denbow, D. M., E. A. Grabau, G. H. Lacy, E. T. Kornegay, D. T. Russell, and P. F. Umbeck. 1998. Soybeans transformed with a fungal phytase genet improve phosphorus availability for broilers. Poult. Sci. 77:878-881. doi:10.1093/ps/77.6.878

Dersjant-Li, Y., Awati, A., Schulze, H., and Partridge, G. 2015. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95:878-896. doi:10.1002/jsfa.6698

Goncalves, M. A. D., S. S. Dritz, M. D. Tokach, J. M. DeRouchey, J. C. Woodworth, and R. D. Goodband. 2016. Fact sheets – comparing phytase sources for pigs and effects of super dosing phytase on growth performance of nursery and finishing pigs. J. Swine Health Prod. 24:97-101

Gourley, K. M., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, and R. D. Goodband. 2018. Determining the available phosphorus release of Natuphos E 5,000 G phytase for nursery pigs. J. Anim. Sci. 96:1101-1107. doi:10.1093/jas/sky006

Gizzi, G., Thyregod, P., Von Holst, C., Bertin, G., Vogel, K., Faurschou-Isaksen, M., Betz, R., Murphy, R., Andersen, B.B. 2008. Determination of phytase activity in feed: Interlaboratory study. J. AOAC Int. 91:259-267

Haefner, S., A. Knietsch, E. Scholten, J. Braun, M. Lohscheidt, and O. Zelder. 2005. Biotechnological production and applications of phytases. Appl. Microbiol. Biotechnol. 68:588-597. doi:10.1007/s00253-005-0005-y

Humer, E., C. Schwarz, and K. Schedle. 2015. Phytate in pig and poultry nutrition. J. Anim. Phys. Anim. Nutr. 99:605-625. doi:10.1111/jpn.12258

Jones, C.K., M.D. Tokach, S.S Dritz, B.W Ratliff, N.L.Horn, R.D. Goodband, J.M. DeRouchey, R. C. Sulabo, and J.L. Nelssen. 2010. Efficacy of different commercial phytase enzymes

and development of an available phosphorus release curve for Escherichia coli-derived phytases in nursery pigs. J. Anim. Sci. 88:3631-3644. doi:10.2527/jas.2010-2936

Lee, J. K., M. E. Duarte, and S. W. Kim. 2017. Super dosing effects of corn-expressed phytase on growth performance, bone characteristics, and nutrient digestibility in nursery pigs fed diets deficient in phosphorus and calcium. J. Anim. Sci. 95(Suppl. 2):144. (Abstr.) doi:10.2527/asasmw.2017.297

Li, S. F., Y. B. Niu, J. S. Liu, L. Lu, L. Y. Zhang, C. Y. Ran, M. S. Feng, B. Du, J. L. Deng, and X. G. Luo. 2013. Energy, amino acid, and phosphorus digestibility of phytase transgenic corn for growing pigs. J. Anim. Sci. 91:298-308. doi:10.2527/jas.2012-5211

Li, X., and R. M. Raab. 2016. Processes for increasing extraction of enzymes from animal feed and measure activity of the same. United States Patent Application US2016/033472

Ligon, J. M., 2016. GraINzyme Phytase. A phytase feed enzyme produced by *Zea mays* expressing a phytase gene derived from *Escherichia coli* K12. GRAS Notice No. AGRN 21, filed with FDA CVM, July 28, 2016, no questions letter received May 23, 2017.

Munoz Alfonso, C. J., L. Blavi, J. N. Broomhead, and H. H. Stein. 2018. Comparison between a novel phytase and a commercial phytase on growth performance and bone measurements in diets fed to growing pigs. J. Anim. Sci. 96(Suppl. 2):147-148. (Abstr.) doi:10.1093/jas/sky073.272

NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Nyannor, E. K., P. Williams, M. R. Bedford, and O. Adeola. 2007. Corn expressing an *Escherichia coli*-derived phytase gene: a proof-of-concept nutritional study in pigs. J. Anim. Sci. 85:1946-1952. doi:10.2527/jas.2007-0037

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ravindran, V., G. Ravindran, and S. Sivalogan. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem. 50:133-136. doi:10.1016/0308-8136(94)90109-0

Rodriguez, E., Porres J. M., Yanming H., and Lei X. G. 1999. Different Sensitivity of Recombinant *Aspergillus niger* Phytase (r-PhyA) and *Escherichia coli* pH 2.5 Acid Phosphatase (r-AppA) to Trypsin and Pepsin *in Vitro.* Arch Biochem Biophys 365:262-257

Rutherford, S. M., T. K. Chung, and P. J. Moughan. 2014. Effect of microbial phytase on phytate P degradation and apparent digestibility of total P and Ca throughout the gastrointestinal tract of the growing pig. J. Anim. Sci. 92:189-197. doi:10.2527/jas2013-6923

RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Selle, P. H., and V. Ravindran. 2007. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99-122. doi:10.1016/j.livsci.2007.05.014

Wang, J., and Kim. W. K. 2020. Evaluation of a novel corn-expressed phytase on growth performance and bone mineralization in broilers fed different levels of dietary nonphytate phosphorus. J. Appl. Poultry Res. 30:100120. doi:10.1016/j.japr.100120

Wensley, M. R., C. M. Vier, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey. 2020. Technical Note: Assessment of two methods for estimating bone ash in pigs. J. Anim. Sci. 98:1-8. doi:10.1093/jas/skaa251

Wensley, M. R., J. M., DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. M. Faser, and B. L. Guo. 2020. Determining the phosphorus release of Smizyme

TS G5 2,500 phytase in diets for nursery pigs. Transl. Anim. Sci. 4:1-9. doi: 10.1093/tas/txaa058

Zhang, Z. B., E. T. Kornegay, J. S. Radcliffe, D. M. Denbow, H. P. Veit, and C. T. Larsen. 2000. Comparison of genetically engineered microbial and plant phytase for young broilers. Poult. Sci. 79:709-717. doi:10.1093/ps/79.5.709

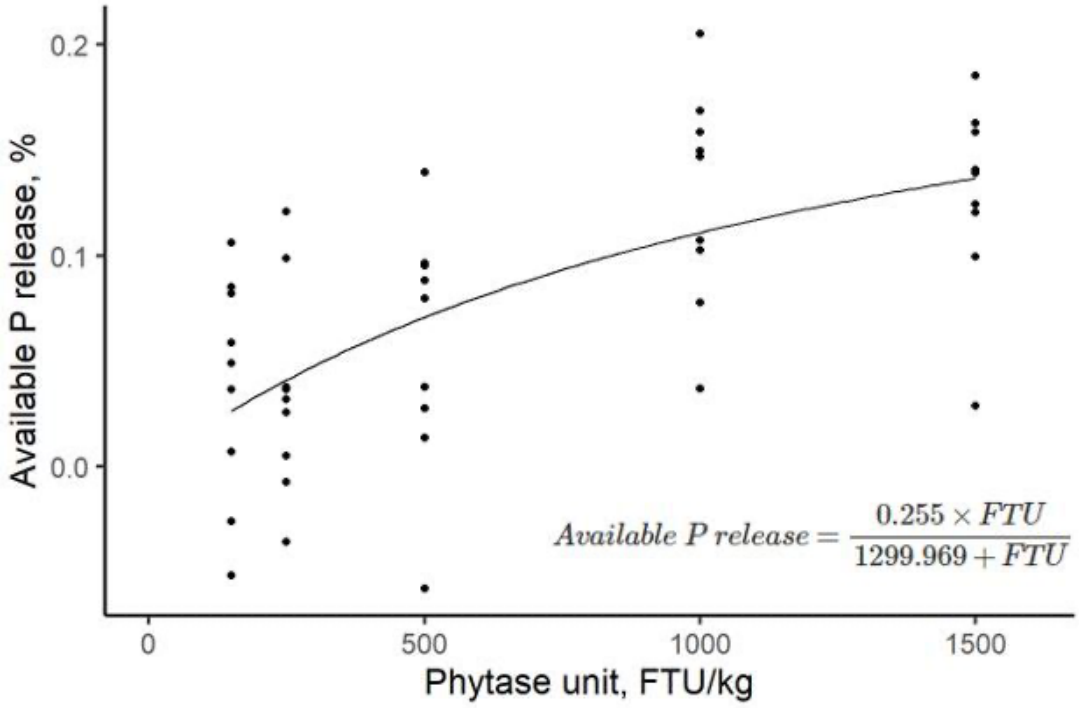

Figure 1.1 Available P release curve for GraINzyme phytase as predicted by ADG.

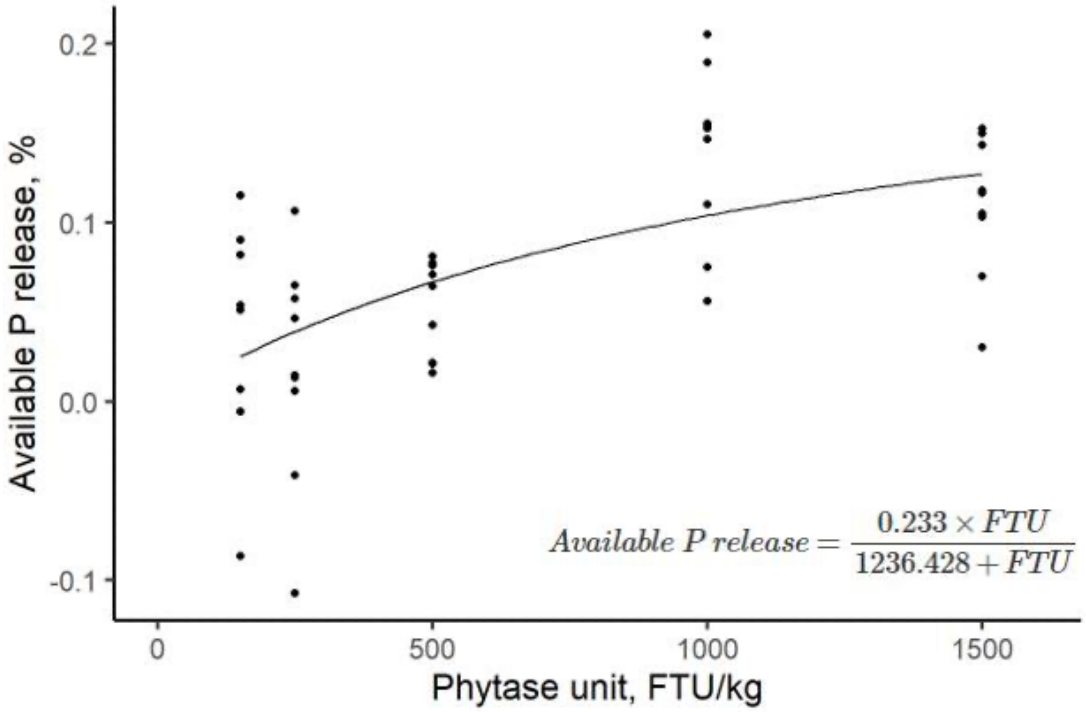

Figure 1.2 Available P release curve for GraINzyme phytase as predicted by G:F.

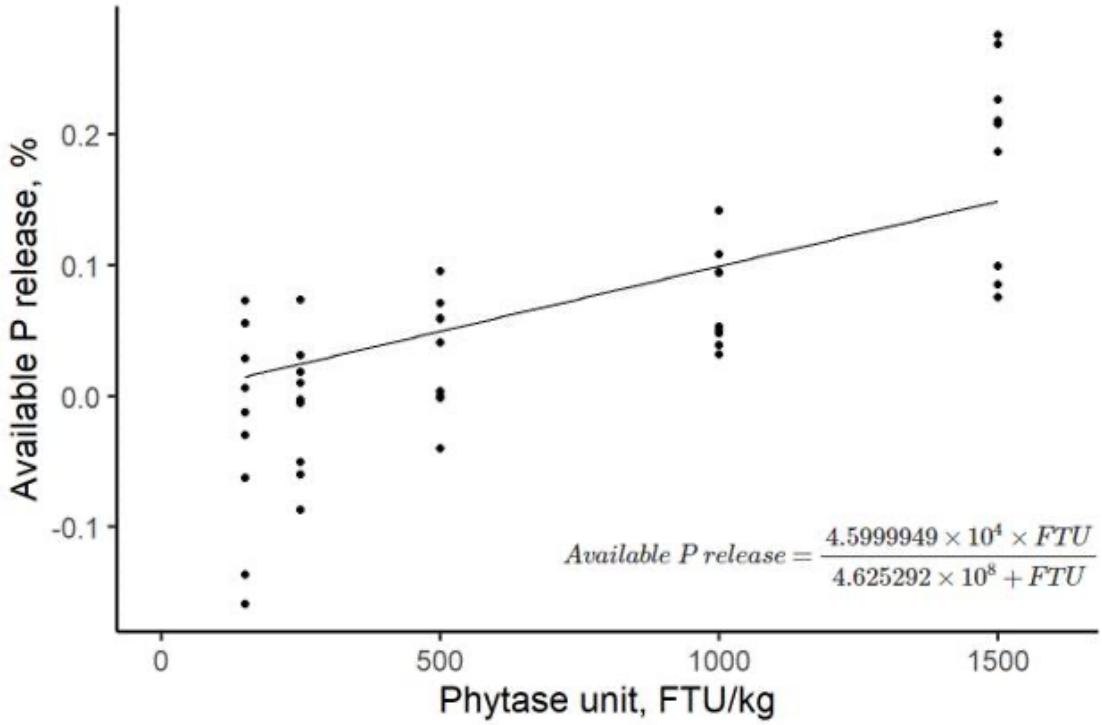

Figure 1.3 Available P release curve for GraINzyme phytase as predicted by bone ash weight (g).

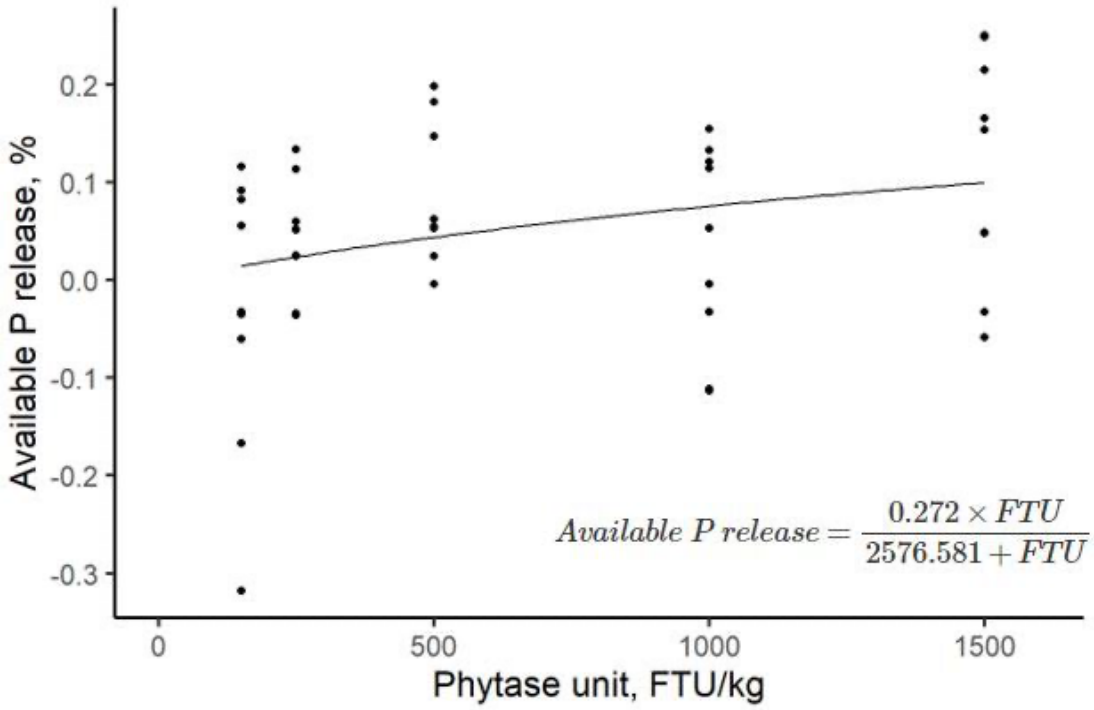

Figure 1.4. Available P release curve for GraINzyme phytase as predicted by bone ash percent.

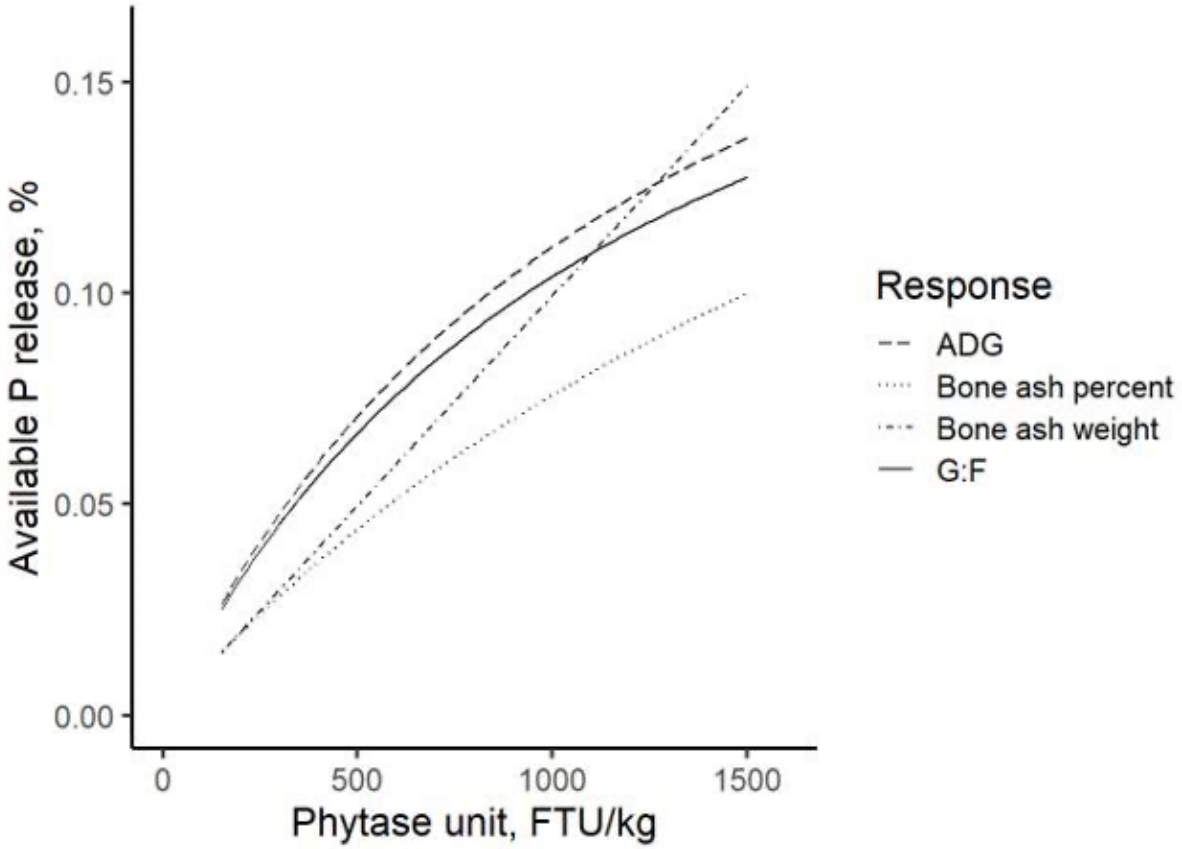

Figure 1.5. Available P release curve for GraINzyme phytase as predicted by ADG, G:F, percentage bone ash, and bone ash weight (g).

Table 1.1 Analyzed ingredient composition (as-fed basis)

Ingredient	Ca, %	P, %
Limestone[1]	38.03	0.10
Monocalcium P[1]	16.73	21.73
Corn[2]	0.03	0.26
Soybean meal[2]	0.38	0.68

[1]Ingredient samples were pooled and analysis was performed by the Kansas State University Soils Lab, Manhattan. Values represent the mean of 3 samples analyzed in duplicate.

[2]Ingredient samples were analyzed by Hubbard Feeds, Beloit, KS.

Table 1.2 Composition of basal mix (as-fed basis)[1]

Item	
Ingredient, %	
Corn	64.35
Soybean meal	34.29
Sodium chloride	0.61
L-Lysine-HCl	0.31
DL-Methionine	0.12
L-Threonine	0.12
L-Valine	0.01
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.05
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lys, %	1.24
Ile:Lys	65
Leu:Lys	131
Met:Lys	34
Met and Cys:Lys	58
Thr:Lys	64
Typ:Lys	19.1
Val:Lys	71
His:Lys	42
Total Lys, %	1.42
Metabolizable energy, kcal/kg	3,338
Net energy (NE), kcal/kg	2,454
SID lysine:NE, g/Mcal	5.05
Crude protein, %	22.1
Ca, %	0.18
P, %	0.40
Available P, %	0.08
STTD P, %[4]	0.17

[1]The basal batch was used as the major ingredient in each experimental diet.

[2]Provided per kg of diet: 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite.

[3]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin.

[4]STTD P = Standardized total tract digestible phosphorus.

Table 1.3 Ingredient composition of experimental diets (as-fed basis)[1]

	Experimental diet							
	Inorganic P			Phytase[2]				
Ingredient, %	0.11	0.19	0.27	150	250	500	1,000	1,500
Basal mix	98.18	98.18	98.18	98.18	98.18	98.18	98.18	98.18
Limestone	0.69	0.77	0.83	0.69	0.69	0.69	0.69	0.69
Monocalcium P	0.16	0.53	0.90	0.16	0.16	0.16	0.16	0.16
Sand[3]	0.98	0.54	0.10	0.97	0.97	0.97	0.96	0.96
Phytase[4]	----	----	----	0.0015	0.0026	0.0051	0.0103	0.0154
Total	100	100	100	100	100	100	100	100
Calculated analysis								
Crude protein, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.47	0.60	0.65	0.47	0.47	0.47	0.47	0.47
P, %	0.43	0.51	0.59	0.43	0.43	0.43	0.43	0.43
Phytase, FTU/kg	----	----	----	150	250	500	1,000	1,500
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[5]								
Ca, %	0.57	0.63	0.77	0.51	0.53	0.59	0.54	0.54
P, %	0.42	0.47	0.63	0.40	0.43	0.43	0.42	0.41
Phytase, FTU/kg[6]	----	----	----	137	223	379	919	1623

[1]Diets were fed for 21 d starting at approximately 10 kg.

[2]GraINzyme (Agrivida Inc., Woburn, MA).

[3]Sand was used to equalize hand-add batch including the addition of limestone, monocalcium P, and phytase when blended with the basal mix.

[4]Phytase was analyzed and contained 9,738,000 FTU/kg (Agrivida Inc., Woburn, MA).

[5]Complete diet samples were taken during bagging of experimental diets from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -20°C until they were submitted for duplicate analysis of Ca and P (Kansas State University Soils Lab, Manhattan).

[6]Five samples of each diet was submitted to Agrivida Inc. (Woburn, MA) for complete phytase analysis using an adjusted extraction procedure that employed an optimized buffer and extraction process.

Table 1.4 Effects of increasing aP from inorganic P or GraINzyme phytase on nursery pig growth performance and bone ash values[1,2]

	Inorganic P, % aP[3]			Phytase, FTU/kg[4]						Inorganic P, *P* <		Phytase, *P* <	
Item	0.11	0.19	0.27	150	250	500	1,000	1,500	SEM	Linear	Quadratic	Linear	Quadratic
BW, kg													
d 0	10.1	10.1	9.8	9.7	9.9	10.1	9.7	10.1	0.19	0.087	0.257	0.229	0.021
d 21	18.7	19.8	20.4	18.9	19.0	19.5	20.0	20.5	0.36	<0.001	0.334	<0.001	0.561
d 0 to 21													
ADG, g	412	465	504	436	434	448	489	491	10.7	<0.001	0.527	<0.001	0.103
ADFI, g	686	739	746	707	717	719	739	757	15.9	0.002	0.151	0.001	0.514
G:F, g/kg	600	630	675	617	606	622	661	649	7.5	<0.001	0.404	<0.001	0.049
Bone characteristics[5]													
Bone ash, g	0.614	0.764	0.812	0.601	0.623	0.672	0.722	0.861	0.0284	<0.001	0.141	<0.001	0.162
Bone ash, %	39.7	43.8	45.4	39.0	41.7	43.0	41.3	44.2	0.01	0.001	0.364	0.003	0.665

[1]A total of 360 nursery pigs (Line 200 × 400, DNA, Columbus, NE; initially 9.9 kg body weight (BW)) were used in a 21-d growth trial to determine the available P (aP) release curve for GraINzyme phytase. There were 5 pigs per pen and 9 replications per treatment.

[2]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

[3]Inorganic P was added to the diet by increasing monocalcium P.

[4]GraINzyme, Agrivida, Woburn, MA.

[5]One pig per pen (9 pens per treatment) was euthanized on d 21 and the right fibula was collected to determine bone ash weight and percentage bone ash. Fibulas were autoclaved for 1 hr. After cleaning, bones were placed in a drying oven at 105°C for 7 days and then ashed in a muffle furnace at 600°C for 24 h.

Table 1.5 Calculated aP release values based on different response criteria[1,2]

	Phytase, FTU/kg[3]						Probability, $P <$	
Item	150	250	500	1,000	1,500	SEM[4]	Linear	Quadratic
Performance								
ADG	0.039	0.035	0.058	0.128	0.129	0.0187	<0.001	0.078
G:F	0.044	0.018	0.052	0.138	0.110	0.0163	<0.001	0.045
Bone characteristics[4]								
Bone ash, g	-0.026	-0.008	0.032	0.074	0.182	0.0195	<0.001	0.138
Bone ash, %	-0.029	0.044	0.083	0.035	0.116	0.0339	0.008	0.721

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: aP intake/day = [dietary aP% - 0.11% (aP in the basal diet)] × ADFI. A standard curve was then developed for each response criterion using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criterion.

[2]ADG = average daily gain. G:F = gain-to-feed ratio.

[3]GraINzyme, Agrivida, Woburn, MA.

[4]One pig per pen (9 pens per treatment) was euthanized on d 21 and the right fibula was collected to determine bone ash weight and percentage bone ash. Fibulas were autoclaved for 1 hr. After cleaning, bones were placed in a drying oven at 105°C for 7 days and then ashed in a muffle furnace at 600°C for 24 h.

Chapter 2 - Effects of standardized ileal digestible lysin on growth performance and economic return in duroc sired finishing pigs

Abstract

In the U.S., emphasis has shifted towards improved pork quality and has resulted in greater use of Duroc-based terminal sires. Duroc sires have differences in ADG, ADFI, G:F, and carcass leanness compared to other sires. Therefore, our objective was to determine the standardized ileal digestible (SID) Lys estimates for Duroc-based sired finishing pigs. In Exp. 1, 2,124 pigs (DNA 600 × PIC 1050, initially 48.9 kg) were used with 24 to 27 pigs per pen and 16 pens per treatment. Corn-soybean meal-based diets were fed in 3 phases (49 to 59, 59 to 71, and 71 to 81 kg). Pens were randomly allotted to 1 of 5 treatments based as a percentage of PIC (2016) SID Lys estimates for gilts (85, 95, 103, 110, and 120%). Phase 1 diets were formulated to 0.90, 1.01, 1.09, 1.17 and 1.27%, phase 2 to 0.79, 0.87, 0.94, 1.03, and 1.10%, and phase 3 to 0.71, 0.78, 0.85, 0.92, and 0.99% SID Lys. Overall, increasing SID Lys increased (linear, $P < 0.001$) ADG and Lys intake/kg of gain. A marginally significant improvement (quadratic, $P = 0.071$) in G:F was observed as SID Lys increased. Feed cost, feed cost/kg of gain, revenue (linear, $P < 0.01$) and income over feed cost (IOFC) increased (quadratic, $P = 0.045$) with increasing SID Lys. In Exp. 2, 2,099 pigs (DNA 600 × PIC 1050, initially 90.1 kg) were used with 24 to 27 pigs per pen and 20 pens per treatment. Corn-soybean meal-based diets were fed in 2 phases (90 to 106 and 106 to 136 kg). Pens were randomly allotted to 1 of 4 treatments based as a percentage of PIC (2016) SID Lys estimates for gilts (85, 93, 100, and 110%). Phase 1 diets were formulated to 0.65, 0.71, 0.77, and 0.84% and phase 2 to 0.60, 0.66, 0.71, and 0.78% SID Lys. Overall, increasing SID Lys increased (linear, $P < 0.05$) market weight, G:F, Lys intake/kg of gain, and increased (quadratic, $P = 0.020$) ADG. Feed cost (linear, $P < 0.01$), revenue, and

IOFC increased (quadratic, $P \leq 0.053$) with increasing SID Lys. In conclusion, the SID Lys estimate for growth and IOFC was 1.19% or 4.63 g of SID Lys/Mcal of NE, 1.05% or 4.04 g of SID Lys/Mcal of NE, and 0.94% or 3.58 g of SID Lys/Mcal of NE for pigs weighing 49 to 59 kg, 59 to 71 kg, and 71 to 81 kg, respectively. The SID Lys estimate for late finishing pigs was 0.74 to 0.81% or 2.85 to 3.10 g of SID Lys/Mcal of NE, and 0.69 to 0.75% or 2.61 to 2.84 g of SID Lys/Mcal of NE, for 90 to 106 kg and 106 to 136 kg pigs, respectively. These data provide SID Lys estimates for current Duroc-based genetic lines raised in a commercial environment.

Key Words: carcass traits, finishing pig, growth, lysine requirement, economics

Introduction

Lysine is typically the first limiting amino acid in corn and soybean meal-based swine diets. In finishing pigs, establishing dietary lysine requirement estimates is crucial for maximizing lean growth and reducing feed cost. There are several factors that impact dietary lysine requirements including: genetics, environmental conditions, sex, and pig body weight (Campbell and Taverner, 1988). Continuous improvements in genetics of modern pigs have resulted in increased growth performance and protein accretion. These may potentially increase amino acid requirement estimates. While genetic suppliers provide amino acid requirement estimates for the various weight ranges of their specific lines, validating these levels is needed within production systems to achieve optimum performance and economic return (De La Llata et al., 2007; Main et al., 2008; Shelton et al., 2011).

Choosing a terminal sire to use in a swine breeding program is important because it influences traits including growth rate, carcass characteristics, pork quality and alters nutrient

requirements. Recently, the U. S. swine industry has placed more emphasis on pork quality resulting in the use of more Duroc-based terminal sires. Duroc's are characterized by their excellent growth rate and intramuscular fat content (Suzuki et al., 2003). Furthermore, Duroc's have a higher feed intake compared to large white pigs (Edwards et al., 1992; Latorre et al., 2003). Soto et al. (2019) utilized a DNA (Line 600, DNA, Columbus, NE) duroc terminal sire and observed a 6% greater overall feed intake compared to PIC genetic lines (Line 337, PIC, Hendersonville, TN) utilized by Goncalves et al. (2017). As a result, nutritional requirements need to be re-evaluated as genetic progress is made over time. Therefore, the objective of these experiments was to determine the optimal standardized ileal digestible lysine requirement for growth performance and economic return of Duroc-based finishing pigs (DNA 600 × PIC 1050) reared in a commercial environment.

Materials and Methods

General

The Pipestone Institutional Animal Care and Use Committee approved the protocols used in these experiments. Both experiments were conducted at a commercial wean-to-finish research facility located in southwest Minnesota (Pipestone Applied Research; Edgerton, MN). Each pen contained one nipple waterer and a 1-hole (two-sided) wet/dry self-feeder or a 4-hole dry self-feeder for *ad libitum* access to feed and water. Treatments were equally allotted and replicated across different feeder types. Pens were located over a completely slatted concrete floor with a deep pit underneath for manure storage. Diets were manufactured at the Spronk Brothers feed mill in Edgerton, MN. Feed was continuously delivered in bulk throughout the study. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen.

Experiment 1

A total of 2,124 barrows and gilts (DNA 600 × PIC 1050, initially 48.9 ± 0.60 kg) were used in a 32-d study. Pens of pigs were blocked by location in the barn and randomly allotted to 1 of 5 dietary treatments with 24 to 27 pig per pen and 16 replications per treatment. A similar number of barrows and gilts were placed in each pen. Diets were fed over 3 phases (49 to 59, 59 to 71, and 71 to 81 kg, respectively) and were corn-soybean meal-based and contained 10% (phase 1 and 2) or 5% (phase 3) dried distillers grains with solubles (DDGS). Ingredient nutrient values and standardized ileal digestibility (SID) coefficients were derived from NRC (2012). Pens were randomly allotted to 1 of 5 dietary treatments [85, 95, 103, 110, and 120% of PIC (2016) gilt recommendations]. The 2016 PIC recommendations on a Lys:cal ME basis were 3.22, 2.77, and 2.46 g of SID Lys/Mcal of ME which corresponds to Lys:cal NE basis of 4.14, 3.56, and 3.15 g of SID Lys/Mcal of NE in phase 1 (49 to 59 kg), phase 2 (59 to 71 kg), and phase 3 (71 to 81 kg), respectively. Phase 1 diets were formulated to contain SID Lys levels of 0.90, 1.01, 1.09, 1.17 and 1.27%, phase 2 levels of 0.79, 0.87, 0.94, 1.03, and 1.10%, and phase 3 levels of 0.71, 0.78, 0.85, 0.92, and 0.99%, respectively. During the experiment, pens of pigs were weighed, and feed disappearance was recorded on d 0, 10, 22, and 32 to determine ADG, ADFI, and G:F.

Experiment 2

A total of 2,099 barrows and gilts (DNA 600 × PIC 1050, initially 90.1 ± 1.69 kg) were used in a 57-d study. Pens of pigs were blocked by location in the barn and randomly allotted to 1 of 4 dietary treatments with 24 to 27 pigs per pen and 20 replications per treatment. A similar number of barrows and gilts were placed in each pen. Diets were fed over 2 phases (90 to 106 and 106 to 136 kg, respectively). Pigs were randomly allotted to 1 of 4 dietary treatments (85,

93, 100, and 110% of PIC [2016] gilt recommendations). The 2016 PIC recommendations on a Lys:cal ME basis were 2.29 and 2.10 g of SID Lys/Mcal of ME which corresponds to Lys:cal NE basis of 2.94, and 2.69 g of SID Lys/Mcal of NE in phase 1 (90 to 106 kg) and phase 2 (106 to 136 kg), respectively. Phase 1 diets were formulated to contain SID Lys levels of 0.65, 0.71, 0.77, and 0.84% and phase 2 levels of 0.60, 0.66, 0.71, and 0.78%, respectively. During the experiment, pens of pigs were weighed, and feed disappearance was recorded on d 0, 16, 29, 44, and 57 to determine ADG, ADFI, and G:F. Pigs were individually ear tagged with RFID ear tags prior to the start of this trial. On d 29 and 44, eight of the heaviest pigs per pen were individually weighed and transported to a commercial packing plant (Wholestone Farms, Fremont, NE) for processing and determination of carcass characteristics. The remaining pigs were marketed on d 57 and transported to the same commercial packing plant for HCW and carcass yield collection.

Economic analysis

Total feed cost per pig, feed cost per kg of gain, revenue, and income over feed cost (IOFC) were calculated for high and low ingredient prices, and market pig price. Feed cost per pig placed was determined by multiplying total feed intake by diet cost. Feed cost per kg of gain was calculated by dividing the total feed cost per pig by the total weight gained. Revenue per pig placed was determined by total gain times the dressing percentage (0.75) and then multiplied by carcass price to convert to a live price. Income over feed cost was calculated using revenue per pig placed minus feed cost per pig placed. For high ingredient price scenarios, the following prices were used: corn = $235.71/tonne ($6.00/bushel); soybean meal = $440/tonne; L-Lys HCl = $1.76/kg; DL-Met = $5.51/kg; L-Thr = $2.65/kg; L-Trp = $11.02/kg; and L-Val = $8.82/kg. For low ingredient price scenarios, the following prices were used: corn = $117.86/tonne

($3.00/bushel); soybean meal = $330/tonne; L-Lys HCl = $1.43/kg; DL-Met = $3.75/kg; L-Thr = $1.87/kg; L-Trp = $6.61/kg; and L-Val = $5.51/kg.

Chemical Analysis

In both experiments, diet samples were collected and sent to a commercial laboratory (Ajinomoto Heartland Inc., Eddyville, IA) for complete amino acid analysis (AOAC 994.12; AOAC, 2006).

Statistical Analysis

In both experiments, data were analyzed as a randomized complete block design with pen as the experimental unit. An initial base model was evaluated using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Treatment was considered a fixed effect and barn location a random effect. Linear and quadratic contrasts were evaluated within increasing SID Lys treatments. Contrast coefficients were adjusted to account for unequal spacing in SID Lys treatments using the IML procedure. Studentized residuals were used to evaluate model assumptions which were reasonably met.

Dose response curves were evaluated using linear (LM), quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models. For each response variable, the best-fitting model was selected using the Bayesian information criterion (BIC), with a lower number indicative of improved fit (Goncalves et al., 2016). Results from both experiments were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Chemical Analysis

In Exp. 1, analyzed total Lys concentration in treatment diets for phases 1 and 2 were similar to formulated values and followed the trend of increasing Lys across treatments (Table

4). In Exp. 2, analyzed total amino acids matched closely with formulated values for treatment diets in phase 1 (Table 5). Phase 3 diet samples from Exp. 1, and phase 2 samples from Exp. 2 were inadvertently not collected and, thus, analysis not available.

Experiment 1

In Exp. 1 (49 to 81 kg barrows and gilts), BW increased linear ($P < 0.01$) on d 10, 22, and 32 (Table 6). Average daily gain increased with increasing SID Lys in phase 1 (linear, $P < 0.001$) and phase 3 (linear, $P < 0.001$; quadratic, $P = 0.022$), resulting in linear ($P < 0.001$) improvement overall. For ADFI, there was a marginally significant linear ($P = 0.067$) increase in phase 1 with increasing SID Lys. A linear ($P \leq 0.035$) improvement for G:F was observed in phase 1 and 2, with the response being linear ($P < 0.001$) and quadratic ($P = 0.009$) in phase 3, leading to a linear ($P < 0.001$) improvement and quadratic ($P = 0.071$) tendency for improvement in the overall period. Daily SID Lys intake and Lys intake per kilogram of BW increased (linear, $P < 0.001$) with increasing dietary Lys.

For economic analysis, feed cost, feed cost per kg gain, and revenue increased linear ($P < 0.01$) as SID Lys increased. Income over feed cost increased (quadratic, $P = 0.045$) with increasing SID Lys. Income over feed cost was greatest when pigs were fed 110% of current SID Lys requirement estimates. Similar findings were observed when using high or low ingredient prices for economic analysis.

Dose response curves were evaluated for overall growth performance and economic analysis. A broken line linear model was the best fitting model for ADG and G:F. Maximum ADG was achieved with a minimum of 112% of current SID Lys requirement estimates (95% CI: 105.3 – 118.8%) with no improvement thereafter (Figure 1A). Similarly, maximum feed efficiency was achieved with a minimum of 112% of current SID Lys requirement estimates

(95% CI: 107.1 – 119.8%; Figure 1B). The BLL model indicated that ADG and G:F were optimized at 112% of current SID Lys requirement estimates, corresponding to diets containing 1.19% SID Lys or 4.63 g of SID Lys/Mcal of NE, 1.05% SID Lys or 4.04 g of SID Lys/Mcal of NE, 0.94% SID Lys or 3.58 g of SID Lys/Mcal of NE in phase 1 (49 to 59 kg), phase 2 (59 to 71 kg), and phase 3 (71 to 81 kg), respectively.

When modeling IOFC, the QP and BLL models had a comparable fit for low and high ingredient prices. The QP model equation developed with high ingredient prices was: IOFC = -2.9232 + 0.465 × (SID Lys%) – 0.00207 × (SID Lys%)^2, with 100% of maximum IOFC estimated at 112.3% SID Lys (Figure 1C). For the BLL with high ingredient prices, maximum IOFC was achieved with a minimum of 105.7% of current SID Lys requirement estimates (95% CI: 93.6 – 117.8%; Figure 1C). The QP model equation developed with low ingredient prices was: IOFC = -4.0787 + 0.3388 × (SID Lys%) – 0.00151 × (SID Lys%)^2, with 100% of maximum IOFC estimated at 112.2% SID Lys (Figure 1D). For the BLL with low ingredient prices, maximum IOFC was achieved with a minimum of 109.2% of current SID Lys requirement estimates (95% CI: 93.5 – 124.9%; Figure 1D). Similar to growth performance, the QP model indicated that IOFC for low and high ingredient prices was optimized at 112% of current SID Lys requirement estimates.

Experiment 2

In Exp. 2 (90 to 136 kg barrows and gilts), d 57 BW and overall market weight increased linearly ($P \leq 0.039$) as SID Lys increased (Table 7). Average daily gain increased in phase 1 (linear, $P < 0.001$) and phase 2 (quadratic, $P < 0.045$), resulting in a quadratic ($P < 0.020$) response overall. For ADFI, there was a marginal significant quadratic ($P \leq 0.092$) increase in phase 1 and the overall period as SID Lys increased up to 93% of current SID Lys requirement

estimates. A linear ($P < 0.001$) improvement in G:F was observed for phase 1 and the overall period with increasing SID Lys in the diet. Additionally, HCW increased linearly ($P = 0.021$) as SID Lys increased. Similar to Exp. 1, daily SID Lys intake and Lys intake per kilogram of BW increased linearly ($P < 0.001$) with increasing dietary Lys.

For economic analysis, feed cost increased linearly ($P < 0.01$) when dietary SID Lys increased. Revenue and income over feed cost increased quadratically ($P \leq 0.02$) when SID Lys increased in the diet with the greatest observed in pigs consuming 100% of current SID Lys requirement estimates, corresponding to pigs fed treatment diets of 0.77% SID Lys or 2.94 g of SID Lys/Mcal of NE in phase 1 (90 to 106 kg) and 0.71% SID Lys or 2.69 g of SID Lys/Mcal of NE in phase 2 (106 to 136 kg). Similar findings were observed when using high or low ingredient prices for economic analysis.

Dose response curves were evaluated for overall growth performance and economic analysis. For ADG, the QP and BLL models had a comparable fit with the SID Lys requirement to achieve maximal performance predicted at 105.6% with QP and 97.0% for the BLL model (Figure 2A). The QP model equation for ADG was: ADG = -1.0914 + 0.04011 × (SID Lys%) – 0.00019 × (SID Lys %)^2. For BLL, there was no further improvement in ADG beyond the breakpoint of 97.0% SID Lys (95% CI: 50.3, 143.8%). For G:F, QP was the best fitting model with the SID Lys requirement to achieve maximal performance predicted at 120.7% SID Lys (Figure 2B). The QP model equation for feed efficiency was: G:F = 0.03976 + 0.004827 × (SID Lys%) – 0.00002 × (SID Lys %)^2. Depending on the model used, growth performance was optimized between 97.0 and 120.7% of current SID Lys requirement estimates, corresponding to diets containing 0.74 to 0.92% SID Lys or 2.85 to 3.53 g of SID Lys/Mcal of NE, and 0.69 to

0.86% SID Lys or 2.61 to 3.25 g of SID Lys/Mcal of NE in phase 1 (90 to 106 kg) and phase 2 (106 to 136 kg), respectively.

When modeling IOFC for low and high ingredient prices, the QP model was the best fit with the SID Lys requirement to achieve maximal IOFC predicted at 99% of the current SID Lys requirement estimates for both high and low ingredient prices, corresponding to diets containing 0.76% SID Lys or 2.85 g of SID Lys/Mcal of NE, and 0.70% SID Lys or 2.66 g of SID Lys/Mcal of NE in phase 1 (90 to 106 kg) and phase 2 (106 to 136 kg), respectively. The QP model equation with high ingredient prices was: IOFC = -59.9429 + 1.6699 × (SID Lys%) – 0.00837 × (SID Lys %)^2 (Figure 2C). The QP model equation with low ingredient prices was: IOFC = -51.4696 + 1.3792 × (SID Lys%) – 0.00694 × (SID Lys %)^2 (Figure 2D).

Discussion

The adoption of new technologies to optimize health status, environmental conditions, management plans, and genomic selection has allowed for significant improvements in pig growth performance and carcass composition over the last 40 years. In 1980, the average market weight was 110 kg (National Pork Board, 2016). By 2019, the average market weight had increased to 128 kg (National Pork Board, 2020). Pigs have also become more feed efficient while being heavier in market weight. In 1990, pigs grew at 0.58 kg/day with 3.2 kg of feed per kg of gain from weaning to market (PigChamp, 1990). In comparison, by 2019, wean-to-finish pigs grew at 0.80 kg/day with 2.6 kg of feed per kg of gain (National Pork Board, 2020). The trends in genetic improvement were also observed in our study as pigs grew at an average of 1.0 kg/day in Exp. 1 (49 to 81 kg pigs) and 0.97 kg/day in Exp. 2 (90 to 136 kg pigs) in a commercial setting. As a result of these genetic advancements, nutrient requirements,

specifically Lys, have increased from 0.66% (NRC, 1998) to 0.85% (NRC, 2012) in 50 to 75 kg pigs.

It has been well documented that factors that change feed intake, such as genetic potential for lean growth (Campbell and Taverner, 1988; Mohn et al., 2000), will influence the dietary SID Lys concentration required to optimize performance. Statistical method of interpreting growth responses (Goncalves et al., 2016) and economics (De La Llata et al., 2001) also play a role in the dietary SID Lys requirement. The degree of feed consumption and associated growth rate may be an important source of variation in estimated SID Lys requirements among experiments. Research conducted with 112 to 136 kg pigs in a university setting, reported an average feed intake of 3.1 kg (Soto et al., 2019). Conversely, research conducted in a commercial environment reported an average feed intake of 2.6 kg for 102 to 120 kg pigs (Main et al., 2008). In agreement, we observed feed intake comparable to other research conducted in a commercial environment in the present study. The level of feed intake and growing environment of the pig have a key impact on SID Lys requirements and further highlight the importance of determining production system-specific nutrient requirements.

Variation in Lys requirements could be attributed to differences in genetic capacities for amino acid utilization by the pig in terms of Lys efficiency and protein deposition. When Lys requirements are expressed as a function of SID Lys required per kg of daily BW gain, our estimated requirement was approximately 24.3 g SID Lys/kg gain for 49 to 81 kg pigs and range from approximately 24.2 to 25.7 g SID Lys/kg gain for 90 to 136 kg pigs. In comparison to Soto et al. (2019) with pigs weighing over 100 kg, our requirement of SID Lys g/kg gain is greater (24.2 to 25.7 vs. 17.0 to 19.8). Furthermore, Main et al. (2008) and Shelton et al. (2011) also observed lower SID Lys g/kg gain requirements of 21.9 and 19.6, respectively. Thus, a greater

requirement of SID Lys intake per kg of BW gain could be credited to the pigs depositing a higher proportion of protein rather than lipids compared to previous research.

Diets are formulated on a lysine-to-calorie ratio, and other amino acids are balanced accordingly as a ratio relative to Lys (Wang and Fuller, 1989). Responses to SID Lys or Lys:calorie ratio should be expected to differ among production environments and genetic lines. This change in diet formulation strategy has been helpful for swine producers through improved growth rate, feed efficiency, and carcass leanness. Thus, the concept of adjusting SID Lys concentration in a ratio relative to dietary energy content has been demonstrated by previous researchers (Allee and Hines, 1972; Chiba et al. 1991; Marcal et al., 2019). Previous research by Soto et al. (2019) reported a Lys:calorie ratio of 2.14 to 2.53 g of SID Lys/Mcal of NE was needed to achieve optimal performance in 100 to 120 kg pigs. We determined the requirement to be a Lys:calorie ratio of 2.85 to 3.10 g of SID Lys/Mcal of NE, and 2.61 to 2.84 g of SID Lys/Mcal of NE in 90 to 106 and 106 to 136 kg pigs, respectively, to achieve optimal economics and growth performance. An accurate requirement estimate for Lys in the finishing growth period becomes crucial for maximizing lean growth and optimizing feed cost leading to a cost-effective nutritional program.

When developing and evaluating nutritional programs, it is crucial to factor in economics along with growth performance. Swine producers can use a variety of economic criteria including income over feed cost which accounts for the revenue and expenses generated. Research has documented that nutrient requirements needed to maximize biological performance may align with optimal IOFC estimates (Main et al., 2008; De La Llata et al., 2001). Similarly, when we evaluated the data in terms of IOFC for both experiments, we observed the dietary SID Lys resulting in optimized growth performance was associated with the optimal IOFC estimate,

even though feed cost was increased. It is documented that feed cost per unit of gain may be more sensitive to changes in feed ingredient prices than IOFC (De La Llata et al., 2001; Main et al., 2008).

In conclusion, the SID Lys requirement to optimize growth performance and IOFC was 1.19% or 4.63 g of SID Lys/Mcal of NE, 1.05% or 4.04 g of SID Lys/Mcal of NE, and 0.94% or 3.58 g of SID Lys/Mcal of NE for pigs weighing 49 to 59 kg, 59 to 71 kg, and 71 to 81 kg, respectively. The SID Lys requirement for late finishing pigs was 0.74 to 0.81% or 2.85 to 3.10 g of SID Lys/Mcal of NE, and 0.69 to 0.75% or 2.61 to 2.84 g of SID Lys/Mcal of NE, for 90 to 106 kg and 106 to 136 kg pigs, respectively. While these requirements are dependent on the statistical model utilized, our data provides SID Lys requirements for current Duroc-based sired pigs grown in a commercial environment. The increased Lys estimate requirements highlight advances in management, health status, and genetic improvements over time.

References

Allee, G. L., and R. H. Hines. 1972. Influence of fat level and calorie:protein ratio on performance of young pigs. J. Anim. Sci. 35:210 (Abstr.). doi:10.2527/jas1972.3551087x

AOAC. 2006. Official methods of analysis AOAC international. 18th ed. Association of Official Analytical Chemists, Arlington, VA.

Campbell, R. G., and M. R. Taverner. 1988. Genotype and sex effects on the relationship between energy intake and protein deposition in growing pigs. J. Anim. Sci. 66:676-686. doi:10.2527/jas1988.663676x

Chiba, L. I., A. J. Lewis, and E. R. Peo. 1991. Amino acid and energy interrelationships in pigs weighing 20 to 50 kilograms: I. Rate and efficiency of weight gain. J. Anim. Sci. 69:694-707. doi:10.2527/1991.692694x

De La Llata, M., S. S. Dritz, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2007. Effects of increasing lysine to calorie ratio and added fat for growing-finishing pigs reared in a commercial environment: I. Growth performance and carcass characteristics. Prof. Anim. Sci. 23:417-428. doi:10.1532/S1080-7446(15)30997-9

De La Llata, M., S. S. Dritz, M. R. Langemeier, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2001. Economics of increasing lysine:calorie ratio and adding dietary fat for growing-finishing pigs reared in a commercial environment. Swine Health Prod. 9:215-223.

Edwards, S. A., J. D. Wood, C. B. Moncrieff, and S. J. Porter. 1992. Comparison of the Duroc and Large White as terminal sire breeds and their effect on pig meat quality. Anim. Prod. 54:289-297. doi:10.1017/S0003356100036928

Gonçalves, M. A. D., U. Orlando, W. Cast, and M. Culberson. 2017. Standardized ileal digestible lysine requirements for finishing PIC pigs under commercial conditions: A meta-analysis. J. Anim. Sci. 95(E. Suppl. 2)E:131-132

Gonçalves, M. A., 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/jas.2015-0106

Latorre, M. A., R. Lazaro, M. I. Gracia, M. Nieto, and G. G. Mateos. 2003. Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight. Meat Sci. 65:1369-1377. doi:10.1016/S0309-1740(03)00059-7

Main, R. G., S. S. Dritz, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2008. Determining an optimum lysine:calorie ratio for barrows and gilts in a commercial finishing facility. J. Anim. Sci. 86:2190-2207. doi:10.2527/jas.2007-0408

Marcal, D. A., C. Kiefer, M. D. Tokach, S. S. Dritz, J. C. Woodworth, R. D. Goodband, H. S. Cemin, and J. M. DeRouchey. 2019. Diet formulation method influences the response to increasing net energy in finishing pigs. Transl. Anim. Sci. 3:1349-1358. doi:10.1093/tas/txz147

Mohn, S., A. M. Gillis, P. H. Moughan, and C. F. M. de Lange. 2000. Influence of dietary lysine and energy intake on body protein deposition and lysine utilization in the growing pig. J. Anim. Sci. 78:1510-1519. doi:10.2527/2000.7861510x

National Pork Board. 2016. Quick facts. National Pork Board, Des Moines, IA. Date accessed: Aug. 2, 2021. Retrieved from: https://porkgateway.org/wp-content/uploads/2015/07/quick-facts-book1.pdf

National Pork Board. 2020. Production Analysis Summary for U.S. Pork Industry: 2017-2019. National Pork Board, Des Moines, IA. Date accessed: Aug. 2, 2021. Retrieved from: https://library.pork.org/media/?mediaId=4D0CDE8D-9898-44F4-A2FB321F87DE331D

NRC. 1998. Nutrient requirements of swine: 10th revised edition. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine: 11th revised edition. Natl. Acad. Press, Washington, DC.

PIC. 2016. Nutrient Specifications Manual. Pig Improvement Company. Hendersonville, TN. Date accessed: Aug. 2, 2021. Retrieved from: https://www.pic.com/wp-content/uploads/sites/3/2018/10/Nutrient-Specifications-Manual_2016_English_Metric.pdf

PigChamp. 1990. Grow-finish production values. Swine Graphics, Inc., Webster City, IA.

Shelton, N. W., M. D. Tokach, S. S. Dritz, R. D. Goodband, J. L. Nelssen, and J. M. DeRouchey. 2011. Effects of increasing dietary standardized ileal digestible lysine for gilts grown in a commercial finishing environment. J. Anim. Sci. 89:3587-3595. doi:10.2527/jas.2010-3030

Soto, J. A., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and F. Wu. 2019. Optimal dietary standardized ileal digestible lysine and crude protein concentration for growth and carcass performance in finishing pigs weighing greater than 100 kg. J. Anim. Sci. 97:1701-1711. doi:10.1093/jas/skz052

Suzuki, K., T. Shibata, H. Kadowaki, H. Abe, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc, and crossbred pigs by Berkshire and Duroc. Meat Sci. 64: 35-42. doi:10.1016/S039-1740(02)00134-1

Wang, T. C., and M. F. Fuller. 1989. The optimum dietary amino acid pattern for growing pigs. Br. J. Nutr. 62:77-89. doi:10.1079/BJN19890009

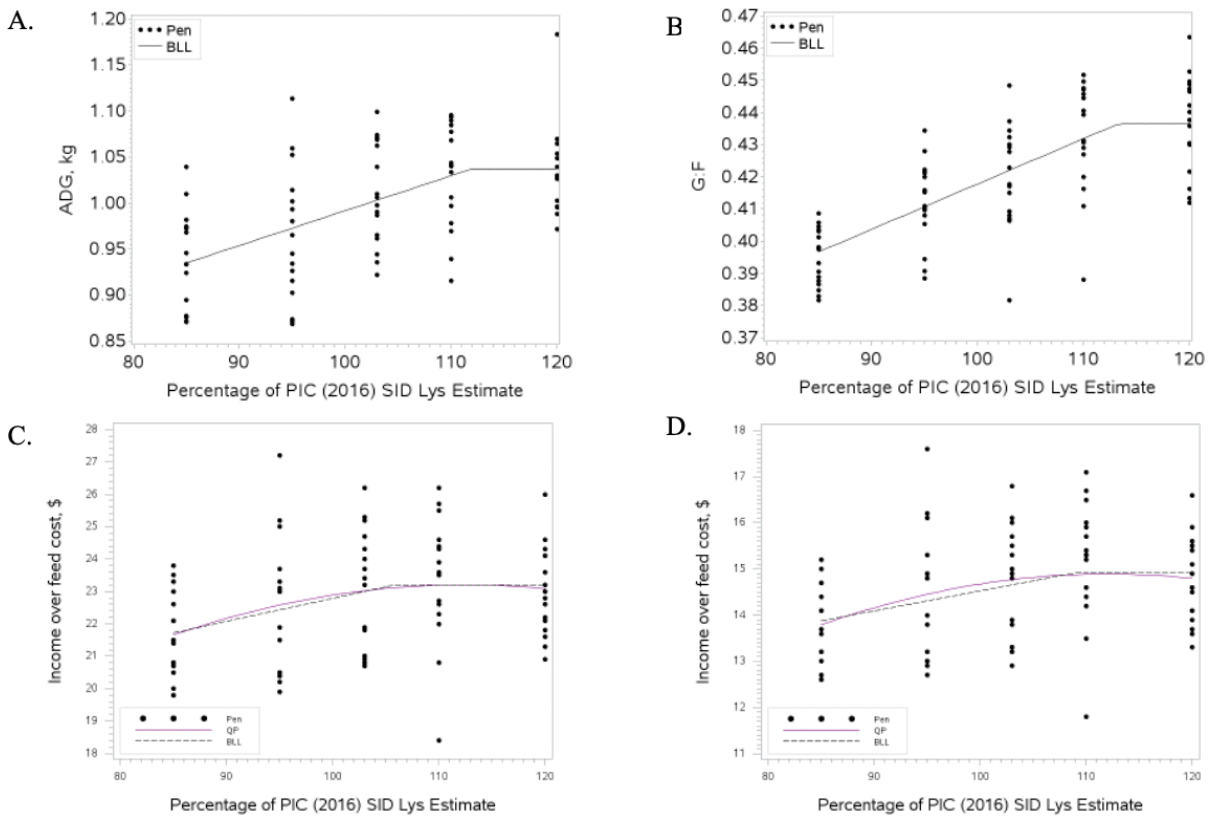

Figure 2.1 Estimation of standardized ileal digestible (SID) lysine requirement to maximize ADG, G:F, and income over feed cost (IOFC) for grow-finish pigs weighing 49 to 81 kg (Exp. 1). A total of 2,124 barrows and gilts (DNA 600 × PIC 1050, initially 48.9 ± 0.60 kg) were used in a 32-d growth trial with 24 to 27 pigs per pen and 16 replications per treatment. A. The broken line linear (BLL) model predicted no further improvement in ADG over 112% of current SID Lys requirement estimates (95% CI: 105.29 – 118.75%) B. The BLL model predicted no further improvement in G:F over 112% of current SID Lys requirement estimates (95% CI: 107.08 – 119.84%) C. The quadratic polynomial (QP) and BLL models had a comparable fit for IOFC. The QP model equation developed with high ingredient prices was: IOFC = -2.9232 + 0.465 × (SID Lys%) – 0.00207 × (SID Lys%)2, with 100% of maximum IOFC estimated at 112.3% SID Lys. For the BLL with high ingredient prices, maximum IOFC was achieved with a minimum of 105.7% of current SID Lys requirement estimates (95% CI: 93.60 – 117.82%). D. The QP and BLL models had a comparable fit for IOFC. The QP model equation developed with low ingredient prices was: IOFC = -4.0787 + 0.3388 × (SID Lys%) – 0.00151 × (SID Lys%)2, with 100% of maximum IOFC estimated at 112.2% SID Lys. For the BLL with low ingredient prices, maximum IOFC was achieved with a minimum of 109.2% of current SID Lys requirement estimates (95% CI: 93.46 – 124.92%).

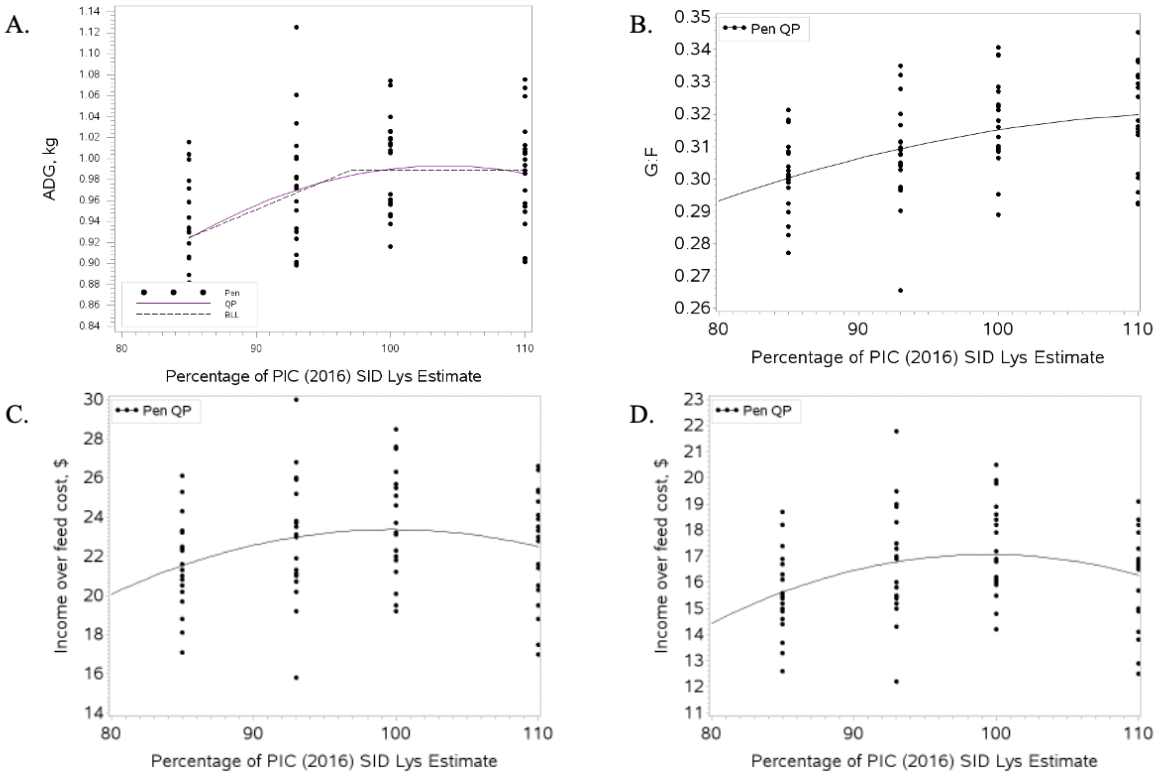

Figure 2.2 Estimation of standardized ileal digestible (SID) Lysine requirement to maximize ADG, G:F, and income over feed cost (IOFC) for grow-finish pigs weighing 90 to 136 kg (Exp. 2). A total of 2,099 pigs (DNA 600 × PIC 1050, initially 90.1 ± 1.69 kg) were used in a 57-d trial with 27 to 24 pigs per pen and 20 replications per treatment. A. The quadratic polynomial (QP) and broken line linear (BLL) models had a comparable fit (BIC = -245.8 and -245.7, QP and BLL, respectively) with the SID Lys requirement to achieve maximal ADG predicted at 105.6% with QP and 97.0% for the BLL model. The QP model equation was: ADG = -1.0914 + 0.04011 × (SID Lys%) – 0.00019 × (SID Lys %)^2. For BLL, there was no further improvement in ADG beyond the breakpoint of 97.0% SID Lys (95% CI: 50.32, 143.75%). B. The QP was the best fitting model with the SID Lys requirement to achieve maximal feed efficiency predicted at 120.7% SID Lys. The QP model equation was: G:F = 0.03976 + 0.004827 × (SID Lys%) – 0.00002 × (SID Lys %)^2. C. The QP model was the best fit with the SID Lys requirement to achieve maximal IOFC with high ingredient prices predicted at 99% of the current SID Lys requirement estimates for high ingredient prices. The QP model equation was: IOFC = -59.9429 + 1.6699 × (SID Lys%) – 0.00837 × (SID Lys %)^2. D. The QP model was the best fit (BIC = 326.98) with the SID Lys requirement to achieve maximal IOFC with low ingredient prices predicted at 99% of the current SID Lys requirement estimates. The QP model equation was: IOFC = -51.4696 + 1.3792 × (SID Lys%) – 0.00694 × (SID Lys %)^2.

Table 2.1 Composition of phase 1 and phase 2 diets in Exp. 1 (as-fed basis)

	Phase 1[2]					Phase 2[3]				
	Percentage of PIC SID Lys estimate[4]									
Item	85	95	103	110	120	85	95	103	110	120
Ingredient, %										
Corn	74.73	69.90	65.96	62.66	57.92	78.54	75.50	72.23	68.68	65.27
Soybean meal, 46.5% CP	11.95	16.35	19.95	22.95	27.25	8.25	11.00	13.95	17.20	20.30
DDGS	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Corn oil	0.78	1.23	1.58	1.88	2.33	0.80	1.08	1.38	1.70	2.03
Monocalcium P, 21% P	0.22	0.19	0.17	0.16	0.13	0.17	0.15	0.14	0.12	0.10
Limestone	0.98	0.95	0.93	0.93	0.90	0.95	0.93	0.93	0.90	0.88
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.49	0.49	0.49	0.49	0.49	0.46	0.48	0.48	0.49	0.49
DL-Met	0.05	0.07	0.10	0.11	0.14	0.02	0.04	0.06	0.08	0.10
L-Thr	0.13	0.14	0.14	0.15	0.16	0.12	0.13	0.14	0.15	0.16
L-Trp	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.04	0.04	0.04
Vitamin and trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Tri-basic copper chloride	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[5]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis[6]										
Standardized ileal digestible (SID) amino acids, %										
Lys	0.90	1.01	1.09	1.17	1.27	0.79	0.87	0.94	1.03	1.10
Ile:Lys	55	57	58	58	59	55	55	56	57	58
Leu:Lys	143	138	134	132	128	152	145	141	137	134
Met:Lys	31	32	33	33	34	29	31	32	32	33
Met and Cys:Lys	58	58	58	58	58	58	58	58	58	58
Thr:Lys	63	63	63	63	63	64	64	64	64	64
Trp:Lys	19	19	19	19	19	19	19	19	19	19

Val:Lys	65	65	65	65	65	66	65	65	65	65
Total Lys, %	1.01	1.13	1.23	1.31	1.42	0.89	0.98	1.06	1.15	1.24
ME, kcal/kg	3,285	3,285	3,285	3,285	3,285	3,307	3,307	3,307	3,307	3,307
NE, kcal/kg	2,557	2,560	2,562	2,564	2,566	2,577	2,582	2,582	2,584	2,586
SID Lys:NE, g/Mcal	3.52	3.93	4.27	4.55	4.96	3.06	3.38	3.65	3.97	4.26
CP, %	14.72	16.42	17.82	18.98	20.65	13.26	15.48	15.48	16.73	17.94
Ca, %	0.58	0.58	0.58	0.58	0.58	0.55	0.55	0.55	0.55	0.55
Available P, %	0.29	0.29	0.29	0.29	0.29	0.28	0.28	0.28	0.28	0.28
STTD P, %	0.35	0.36	0.37	0.37	0.38	0.34	0.34	0.34	0.35	0.35

[1]Treatment diets were fed to 2,124 pigs (DNA 600 × PIC 1050, initially 48.9 ± 0.60 kg).

[2]Phase 1 treatment diets were fed from 49 to 59 kg.

[3]Phase 2 treatment diets were fed from 59 to 71 kg.

[4]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[5]Quantum Blue 5G (AB Vista, Marlborough, UK) provided an estimated release of 0.12% available P.

[6]Ingredient values and SID coefficients were derived from NRC 2012 Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2.2 Composition of phase 3 diets in Exp. 1 (as-fed basis)[1,2]

	Percentage of PIC SID Lys estimate[3]				
Item	85	95	103	110	120
Ingredient, %					
Corn	83.98	80.81	77.70	74.50	71.27
Soybean meal, 46.5% CP	7.65	10.55	13.40	16.30	19.25
DDGS	5.00	5.00	5.00	5.00	5.00
Corn oil	1.10	1.40	1.68	1.98	2.28
Monocalcium P, 21% P	0.20	0.19	0.17	0.15	0.14
Limestone	0.88	0.85	0.83	0.83	0.80
Sodium chloride	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.40	0.40	0.40	0.40	0.40
DL-Met	0.00	0.02	0.03	0.05	0.07
L-Thr	0.10	0.10	0.11	0.12	0.12
L-Trp	0.04	0.03	0.03	0.03	0.03
Vitamin and trace mineral premix	0.10	0.10	0.10	0.10	0.10
Tri-basic copper chloride	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.04	0.04	0.04	0.04	0.04
Total	100	100	100	100	100
Calculated analysis[5]					
Standardized ileal digestible (SID) amino acids					
Lys, %	0.71	0.78	0.85	0.92	0.99
Ile:Lys	56	57	58	58	58
Leu:Lys	154	148	144	140	137
Met:Lys	28	29	30	31	31
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	64	64	64	64	64
Trp:Lys	19	19	19	19	19
Val:Lys	67	67	67	67	67
Total Lys, %	0.80	0.87	0.95	1.03	1.11
ME, kcal/kg	3,344	3,344	3,344	3,344	3,344
NE, kcal/kg	2,612	2,615	2,615	2,617	2,619
SID Lys:NE, g/Mcal	2.72	2.99	3.25	3.52	3.79
CP, %	12.04	13.16	14.26	15.39	16.53
Ca, %	0.52	0.52	0.52	0.52	0.52
Available P, %	0.26	0.26	0.26	0.26	0.26
STTD P, %	0.33	0.33	0.33	0.34	0.34

[1]Treatment diets were fed to 2,124 pigs (DNA 600 × PIC 1050, initially 48.9 ± 0.60 kg).

[2]Phase 3 treatment diets were fed from 71 to 81 kg.

[3]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[4]Quantum Blue 5G (AB Vista, Marlborough, UK) provided an estimated release of 0.12% available P.

[5]Ingredient values and SID coefficients were derived from NRC 2012 Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2.3 Composition of Exp.2 treatment diets (as-fed basis)[1]

	Phase 1[2]				Phase 2[3]			
	Percentage of PIC SID Lys estimate[4]							
Item	85	93	100	110	85	93	100	110
Ingredient, %								
Corn	90.36	88.16	85.89	82.43	89.99	87.58	85.34	82.11
Soybean meal, 46.5% CP	6.85	8.85	10.90	14.05	7.05	9.25	11.30	14.25
Corn oil	0.65	0.85	1.05	1.38	0.93	1.18	1.38	1.68
Monocalcium P, 21% P	0.21	0.20	0.19	0.18	0.21	0.20	0.19	0.17
Limestone	0.78	0.75	0.75	0.73	0.78	0.75	0.75	0.73
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.37	0.39	0.39	0.39	0.30	0.30	0.30	0.30
DL-Met	0.00	0.01	0.03	0.05	0.00	0.00	0.00	0.01
L-Thr	0.10	0.11	0.12	0.13	0.07	0.07	0.08	0.09
L-Trp	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02
Vitamin and trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Tri-basic copper chloride	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[5]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Total	100	100	100	100	100	100	100	100
Calculated analysis[6]								
Standardized ileal digestible (SID) amino acids, %								
Lys	0.65	0.71	0.77	0.84	0.60	0.66	0.71	0.78
Ile:Lys	55	55	56	57	60	61	60	62
Leu:Lys	151	145	141	136	164	159	154	149
Met:Lys	27	28	29	30	30	29	29	27
Met and Cys:Lys	59	58	58	58	64	62	60	58
Thr:Lys	65	65	65	65	66	66	66	66
Trp:Lys	19	19	19	19	19	19	19	19
Val:Lys	66	65	65	65	73	72	71	71

Total Lys, %	0.72	0.79	0.85	0.93	0.65	0.73	0.79	0.86
ME, kcal/kg	3,351	3,351	3,351	3,351	3,362	3,362	3,362	3,362
NE, kcal/kg	2,610	2,610	2,612	2,615	2,621	2,626	2,626	2,628
SID Lys:NE, g/Mcal	2.50	2.73	2.94	3.22	2.30	2.50	2.69	2.96
CP, %	10.81	11.59	12.39	13.61	10.84	11.69	12.49	13.64
Ca, %	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Available P, %	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.25
STTD P, %	0.31	0.31	0.32	0.32	0.31	0.31	0.32	0.32

[1]Treatment diets were fed to 2,099 pigs (DNA 600 × PIC 1050, initially 90.1 ± 1.69 kg).

[2]Phase 1 treatment diets were fed from 90 to 106 kg.

[3]Phase 2 treatment diets were fed from 106 to 136 kg.

[4]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[5]Quantum Blue 5G (AB Vista, Marlborough, UK) provided an estimated release of 0.12% available P.

[6]Ingredient values and SID coefficients were derived from NRC 2012 Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2.4 Chemical analysis of diets in Exp.1 (as-fed basis)[1,2]

	Percentage of PIC SID Lys estimate[3]				
Item	85	95	103	110	120
Phase 1[4]					
Proximate analysis, %					
DM	87.18	87.46	87.59	87.66	87.92
CP	14.7	16.7	18.5	19.6	21.7
Amino acid analysis, %					
Lys	1.07 (1.01)	1.27 (1.13)	1.39 (1.23)	1.44 (1.31)	1.61 (1.44)
Ile	0.55 (0.58)	0.63 (0.66)	0.72 (0.72)	0.76 (0.78)	0.87 (0.86)
Leu	1.42 (1.48)	1.58 (1.60)	1.69 (1.69)	1.77 (1.77)	1.92 (1.88)
Met	0.27 (0.32)	0.31 (0.36)	0.36 (0.40)	0.38 (0.43)	0.44 (0.48)
Thr	0.64 (0.66)	0.74 (0.74)	0.83 (0.80)	0.88 (0.86)	0.97 (0.94)
Trp	0.18 (0.19)	0.19 (0.21)	0.23 (0.23)	0.24 (0.24)	0.28 (0.27)
Val	0.69 (0.70)	0.79 (0.78)	0.94 (0.84)	0.99 (0.90)	1.11 (0.97)
Phase 2[5]					
Proximate analysis, %					
DM	87.36	87.34	87.15	87.37	87.41
CP	15.0	14.8	15.8	17.1	18.1
Amino acid analysis, %					
Lys	1.11 (0.89)	1.14 (0.98)	1.19 (1.06)	1.22 (1.15)	1.28 (1.24)
Ile	0.56 (0.51)	0.55 (0.56)	0.59 (0.61)	0.63 (0.67)	0.69 (0.73)
Leu	1.46 (1.39)	1.46 (1.46)	1.48 (1.53)	1.56 (1.61)	1.65 (1.69)
Met	0.28 (0.27)	0.28 (0.31)	0.30 (0.34)	0.33 (0.37)	0.35 (0.40)
Thr	0.66 (0.59)	0.68 (0.65)	0.71 (0.71)	0.76 (0.76)	0.80 (0.82)
Trp	0.18 (0.16)	0.18 (0.18)	0.20 (0.20)	0.21 (0.21)	0.23 (0.23)
Val	0.71 (0.63)	0.71 (0.68)	0.79 (0.73)	0.85 (0.79)	0.92 (0.85)

[1]Diet samples were taken from 1 feeder per treatment and submitted to Ajinomoto Heartland Inc. (Eddyville, IA) for amino acid analysis.

[2]Numbers in parenthesis are the formulated values.

[3]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[4]Phase 1 treatment diets were fed from 49 to 59 kg.

[5]Phase 2 treatment diets were fed from 59 to 71 kg.

Table 2.5 Chemical analysis of phase 1 diets in Exp. 2 (as-fed basis)[1,2]

	Percentage of PIC SID Lys estimate[3]			
Item	85	93	100	110
Proximate analysis, %				
DM	86.18	86.71	86.70	86.77
CP	10.0	11.5	12.3	13.2
Total amino acid analysis, %				
Lys	0.67 (0.72)	0.72 (0.79)	0.84 (0.85)	0.94 (0.93)
Ile	0.32 (0.40)	0.40 (0.44)	0.45 (0.47)	0.50 (0.53)
Leu	0.94 (1.12)	1.07 (1.17)	1.17 (1.22)	1.23 (1.30)
Met	0.17 (0.21)	0.20 (0.23)	0.22 (0.25)	0.28 (0.29)
Thr	0.44 (0.47)	0.51 (0.52)	0.56 (0.56)	0.61 (0.62)
Trp	0.12 (0.13)	0.13 (0.14)	0.15 (0.15)	0.17 (0.17)
Val	0.46 (0.50)	0.53 (0.53)	0.58 (0.57)	0.61 (0.63)

[1]Diet samples were taken from 1 feeder per treatment and submitted to Ajinomoto Heartland Inc. (Eddyville, IA) for amino acid analysis. Phase 1 treatment diets were fed from 90 to 106 kg.

[2]Numbers in parenthesis are the formulated values.

[3]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

Table 2.6 Effects of increasing standardized ileal digestible (SID) lysine on grow-finish pig performance in Exp. 1[1]

	Percentage of PIC SID Lys estimate[2]							
	85	95	103	110	120			
Phase 1 SID Lys, %	0.90	1.01	1.09	1.17	1.27			
Phase 2 SID Lys, %	0.79	0.87	0.94	1.03	1.10			
Phase 3 SID Lys, %	0.71	0.78	0.85	0.92	0.99		Probability, *P* <	
Item						SEM	Linear	Quadratic
BW, kg								
d 0	48.9	49.0	49.0	48.9	48.8	0.60	0.875	0.672
d 10	57.8	57.7	59.2	59.2	59.1	0.64	0.007	0.510
d 22	69.8	70.0	71.4	71.4	71.9	0.66	0.001	0.733
d 32	78.9	80.0	81.3	81.9	82.0	0.72	< 0.001	0.213
d 0 to 10 (phase 1)								
ADG, kg	0.894	0.864	1.020	1.032	1.034	0.0242	< 0.001	0.506
ADFI, kg	2.105	1.933	2.192	2.167	2.142	0.0583	0.067	0.812
G:F	0.427	0.455	0.467	0.478	0.486	0.0128	0.001	0.370
d 10 to 22 (phase 2)								
ADG, kg	1.003	1.029	1.016	1.015	1.063	0.0235	0.104	0.471
ADFI, kg	2.446	2.495	2.482	2.448	2.456	0.0350	0.816	0.286
G:F	0.408	0.413	0.409	0.415	0.434	0.0079	0.035	0.205
d 22 to 32 (phase 3)								
ADG, kg	0.904	0.984	0.988	1.044	1.010	0.0290	0.001	0.022
ADFI, kg	2.536	2.551	2.513	2.519	2.521	0.0484	0.575	0.905
G:F	0.356	0.386	0.393	0.415	0.401	0.0096	< 0.001	0.009
d 0 to 32								
ADG, kg	0.939	0.964	1.008	1.030	1.037	0.0145	< 0.001	0.127
ADFI, kg	2.376	2.336	2.400	2.382	2.378	0.0338	0.350	0.806
G:F	0.395	0.412	0.421	0.434	0.437	0.0038	< 0.001	0.071
Lys intake g/d	18.9	20.4	22.9	24.6	26.5	0.41	< 0.001	0.923
Lys intake g/kg gain	20.1	21.2	22.8	23.9	25.5	0.28	< 0.001	0.292
Removals, %	0.47	0.24	0.23	0.71	0.70	0.407	0.416	0.404
Mortality, %	0.47	0.47	0.93	0.47	0.47	0.465	0.995	0.615
Total Removals, %	0.95	0.71	1.17	1.18	1.17	0.524	0.555	0.939
Economics, $/pig placed								
Low ingredient prices[3]								
Feed cost	15.88	16.14	17.26	17.29	18.06	0.251	< 0.001	0.887
Feed cost/kg gain[4]	0.531	0.525	0.538	0.530	0.547	0.0047	0.007	0.073
Revenue[5]	29.71	30.56	31.87	32.44	32.79	0.469	< 0.001	0.149
IOFC[6]	13.84	14.40	14.61	15.14	14.71	0.298	0.001	0.045
High ingredient prices[7]								

Feed cost	21.85	22.39	23.75	24.19	25.08	0.347	< 0.001	0.961
Feed cost/kg gain	0.731	0.728	0.740	0.741	0.759	0.0065	0.001	0.115
Revenue[8]	43.59	44.79	46.75	47.58	48.08	0.689	< 0.001	0.149
IOFC	21.73	22.43	23.01	23.38	23.01	0.447	0.001	0.064

[1]A total of 2,124 pigs (DNA 600 × PIC 1050, initially 48.9 ± 0.60 kg) were used with 27 to 24 pigs per pen and 16 replications per treatment in a 32-d study. Phase 1 treatment diets were fed from 49 to 59 kg. Phase 2 diets were fed from 59 to 71 kg. Phase 3 diets were fed from 71 to 81 kg.

[2]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[3] Corn = $117.86/tonne ($3.00/bushel); soybean meal = $330/tonne; L-Lys HCl = $1.43/kg; DL-Met = $3.75/kg; L-Thr = $1.87/kg; L-Trp = $6.61/kg; and L-Val = $5.51/kg.

[4]Feed cost/kg gain = total feed cost per pen ÷ total gain per pen.

[5]Revenue = (total gain/pig placed × 0.75) × $1.32/kg.

[6]Income over feed cost = revenue – feed cost.

[7]Corn = $235.71/tonne ($6.00/bushel); soybean meal = $440/tonne; L-Lys HCl = $1.76/kg; DL-Met = $5.51/kg; L-Thr = $2.65/kg; L-Trp = $11.02/kg; and L-Val = $8.82/kg.

Corn = $6.00/bushel ($232.14/ton); soybean meal = $400/ton; L-Lys HCl = $1.76/kg; DL-Met = $5.51/kg; L-Thr = $2.65/kg; L-Trp = $11.02/kg; L-Val = $8.82/kg

[8]Revenue = (total gain/pig placed × 0.75) × $1.94/kg.

Table 2.7 Effects of increasing standardized ileal digestible (SID) lysine on grow-finish pig performance in Exp. 2[1]

	Percentage of PIC SID Lys estimate[2]						
	85	93	100	110			
Phase 1 SID Lys, %	0.65	0.71	0.77	0.84			
Phase 2 SID Lys, %	0.60	0.66	0.71	0.78		Probability, *P* <	
Item					SEM	Linear	Quadratic
BW, kg							
d 0	89.9	90.0	90.0	90.2	1.69	0.766	0.961
d 16	104.9	105.8	106.1	106.7	1.22	0.107	0.759
d 57[3]	132.8	135.4	137.1	137.0	1.69	0.029	0.275
Market weights							
Cut 1[4] (d 29)	125.5	126.6	127.5	127.1	1.31	0.240	0.436
Cut 2[4] (d 44)	134.6	137.6	137.6	138.3	1.72	0.007	0.166
Overall market weight	131.2	133.5	133.8	134.1	1.54	0.039	0.267
d 0 to 16 (phase 1)							
ADG, kg	0.926	0.981	1.000	1.027	0.0376	< 0.001	0.250
ADFI, kg	2.834	2.936	2.919	2.880	0.0364	0.509	0.057
G:F	0.327	0.334	0.343	0.357	0.0146	< 0.001	0.627
d 16 to 57 (phase 2)							
ADG, kg	0.925	0.960	0.988	0.961	0.0162	0.087	0.045
ADFI, kg	3.213	3.268	3.259	3.208	0.0521	0.842	0.224
G:F	0.276	0.280	0.287	0.283	0.0048	0.193	0.376
d 0 to 57							
ADG, kg	0.925	0.967	0.992	0.985	0.0148	0.001	0.020
ADFI, kg	3.074	3.148	3.135	3.089	0.0352	0.883	0.092
G:F	0.301	0.308	0.317	0.319	0.0032	< 0.001	0.355
Lysine intake g/d	20.1	22.4	24.0	26.0	0.27	< 0.001	0.112
Lysine intake g/kg gain	21.7	23.2	24.3	26.5	0.26	< 0.001	0.493
Total removals, %	2.46	3.05	1.72	2.65	0.800	0.845	0.634
Carcass performance[5]							
HCW, kg	97.0	98.5	99.1	99.5	0.92	0.021	0.419
Yield, %	73.94	73.75	74.11	74.19	0.003	0.145	0.533
Economics, $/pig placed							
Low ingredient prices[6]							
Feed cost	24.53	25.58	26.26	26.71	0.307	< 0.001	0.194
Feed cost/kg gain[7]	0.606	0.602	0.601	0.616	0.007	0.281	0.153
Revenue[8]	40.22	42.30	43.41	43.00	0.705	0.001	0.011
IOFC[9]	15.67	16.71	17.14	16.27	0.510	0.293	0.020

High ingredient prices[10]							
Feed cost	37.31	39.33	40.06	40.65	0.482	< 0.001	0.070
Feed cost/kg gain	0.922	0.925	0.918	0.939	0.0097	0.282	0.358
Revenue[11]	58.98	62.02	63.67	63.04	1.029	0.001	0.011
IOFC	21.64	22.70	23.62	22.43	0.728	0.290	0.053

[1]A total of 2,099 pigs (DNA 600 × PIC 1050, initially 90.1 ± 1.69 kg) were used with 27 to 24 pigs per pen and 20 replications per treatment. Phase 1 diets were fed from 90 to 106 kg. Phase 2 diets were fed from 106 to 136 kg.

[2]Columns represent the percentage of the 2016 PIC SID Lys recommendations for gilts.

[3]Values represent weights at final barn dump.

[4]Eight of the heaviest pigs were marketed from each pen.

[5]Carcass data were collected from all pigs.

[6]Corn = $117.86/tonne ($3.00/bushel); soybean meal = $330/tonne; L-Lys HCl = $1.43/kg; DL-Met = $3.75/kg; L-Thr = $1.87/kg; L-Trp = $6.61/kg; L-Val = $5.51/kg.

[7]Feed cost/kg gain = total feed cost per pen ÷ total gain per pen.

[8]Revenue = (total gain/pig placed × 0.75) × $1.32/kg.

[9]Income over feed cost = revenue – feed cost.

[10]Corn = $235.71/tonne ($6.00/bushel); soybean meal = $440/tonne; L-Lys HCl = $1.76/kg; DL-Met = $5.51/kg; L-Thr = $2.65/kg; L-Trp = $11.02/kg; L-Val = $8.82/kg

[11]Revenue = (total gain/pig placed × 0.75) × $1.94/kg.

Chapter 3 - Evaluation of dietary mycotoxin control strategies on nursery pig growth performance and blood measures

Abstract

A total of 4,318 pigs (337 × 1050, PIC; initially 6.5 ± 0.08 kg) were used in a 35-d study to evaluate dietary mycotoxin control strategies on nursery pig performance and blood measures. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments in a randomized complete block design with blocking structure including sow farm origin, date of entry into facility, and average pen BW. A total of 160 pens were used with 80 double-sided 5-hole stainless steel fence line feeders, with feeder serving as the experimental unit. For each feeder, 1 pen contained 27 gilts and 1 pen contained 27 barrows. There were 16 replications per dietary treatment. A common phase 1 diet was fed to all pigs in pelleted form for 7 d prior to treatment diets. Experimental treatments were fed from d 7 to 42 after weaning (d 0 to 35 of the study) and included a low deoxynivalenol (DON) diet (1.12 ± 0.623 mg/kg), high DON diet (2.34 ± 1.809 mg/kg), high DON+ 0.50% sodium metabisulfite (SMB), high DON+ one of two mitigating products; 0.30% Technology1, or 0.30% Technology1+. Technology1 and 1+ are comprised of clays, yeast cell wall components and a blend of plant extracts. Technology1+ also contains SMB. Overall (d 0 to 35), pigs fed high DON had decreased ($P <$ 0.05) final BW, ADG, and ADFI compared to low DON. Additionally, pigs fed high DON+SMB had increased ($P < 0.05$) ADG compared to all other treatments. An improvement ($P < 0.05$) in G:F was observed in pigs fed high DON+SMB or high DON+Technology1+ compared to the low DON or high DON+Technology1 diets with high DON diets intermediate. Pigs fed high DON+SMB or high DON+Technology1 diets had reduced ($P < 0.05$) total removals and

mortality compared to pigs fed low DON diets with high DON and high DON+Technology1+ intermediate. Liquid chromatography with tandem mass spectrometry analysis of dried blood spots collected on d 35 revealed that pigs fed high DON or high DON+Technology1 had increased ($P < 0.05$) DON concentrations compared to low DON with high DON+SMB and high DON+Technology1+ intermediate. In summary, pigs fed high DON diets had reduced performance compared to pigs fed low DON. Sodium metabisulfite supplementation to high DON diets provided a benefit in growth performance with ADG and G:F exceeding growth performance in the low DON diet, with other metabolic changes associated with Technology1+ warranting further investigation.

Key Words: Deoxynivalenol, mycotoxin, nursery pigs, sodium metabisulfite

Introduction

Mycotoxins are naturally occurring secondary metabolites produced by species of molds that commonly contaminate agricultural crops worldwide. Mycotoxins are produced from mold growth during different stages of food and feed production. Mold growth can be divided into 2 categories: field molds and storage molds. Field molds infect crops as parasites and mainly contaminate seeds and plants in the field. Storage molds grow in feedstuffs after harvest during grain storage.

The *Fusarium* species is classified as a field mold and can produce trichothecenes. Deoxynivalenol (DON) is a type B trichothecene that interferes with protein synthesis, impacts immunity, and can cause organ damage (Ehrlich and Diagle, 1987; Pestka and Smolinski, 2005). Pigs are the most susceptible livestock species to DON with exposure to concentrations greater

than 1 mg/kg resulting in decreased feed intake and overall growth (Dänicke et al., 2001). Higher concentrations can result in complete feed refusal and in certain circumstances can cause vomiting (Forsyth et al., 1977; Rotter et al., 1996; Eriksen and Pattersson, 2004).

There are several strategies of detoxification available to alleviate DON effects in swine diets (Weaver et al., 2014; Frobose et al., 2015; Shawk et al., 2019). Contaminated feed can be treated chemically (sodium metabisulfite [SMB] mixed with heat and moisture as well as supplemented in diets; Frobose et al., 2015; Shawk et al., 2019) or biologically in which probiotics or enzymes are used to limit DON effects during digestion (Cheng et al., 2006). It has been well documented that SMB and SMB-based feed additives included in swine diets contaminated with DON result in improved growth performance (Patience et al., 2014; Frobose et al., 2015; Shawk et al., 2019).

Although no feed additives are approved as DON-detoxifying agents by the U.S. Food and Drug Administration, some products have been shown to be beneficial, but more research is needed to compare their effectiveness. Therefore, the objective of this trial was to determine the effect of in-feed technologies in DON-contaminated diets on growth performance, serum chemistry and enzymatic activity, and hematological outcomes in nursery pigs.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this study. This study was conducted at a commercial research-facility located in north central Ohio (Bucyrus, OH). A total of 160 pens were used with 80 double-sided 5-hole stainless steel fence line feeders each feeding 2 adjacent pens with feeder serving as the experimental unit. For each feeder, 1 pen contained 27 gilts and 1 pen contained 27 barrows.

There were 16 replications per dietary treatment. Each pen was also equipped with a cup-waterer to provide *ad libitum* access to feed and water. Feed additions to each individual feeder were made and recorded by an electronic feeding system (Dry Exact; Big Dutchman, Inc. Holland, MI).

Animals and Housing

A total of 4,318 pigs (337 × 1050, PIC, Hendersonville, TN; initially 6.5 ± 0.08 kg) were used in a 35-d growth study. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments in a randomized complete block design with blocking structure including sow farm of origin, date of entry into the facility, and average pen BW. A common phase 1 diet was fed in pelleted form to all pigs for approximately 7 d immediately after weaning and prior to treatment diets. Experimental treatments included a low deoxynivalenol (DON) diet (1.12 ± 0.623 mg/kg), a high DON diet (2.34 ± 1.809 mg/kg), high DON+sodium metabisulfite (SMB), high DON+Technology1, or high DON+Technology1+. Sodium metabisulfite was included at a rate of 0.50% of the diet (Table 1). Technology1 and Technology1+ (Innovad Global; Essen, Belgium) were included at 0.30% of the diet. Technology1 and 1+ are comprised of clays, yeast cell wall components, and a blend of plant extracts. Technology1+ also contains SMB. Treatment diets were fed from d 7 to 42 after weaning (d 0 to 35 of the study) and were manufactured at the Hord Elevator (Bucyrus, OH). All nutrients met NRC (2012) requirement estimates.

All feed used in this experiment was manufactured in a commercial feed mill that receives loads of corn weekly. The facility uses a rapid test to evaluate the level of DON in each incoming load of corn and segregates into low and high DON storage bins for segregated use within diets fed to different phases of production. Low and high DON corn samples were

collected during the study including 1 set of samples at the beginning of the study and 1 set of samples at the conclusion of the study. Complete feed samples were collected from at least 6 feeders per treatment per feed delivery (approximately 4 to 5 deliveries depending on the treatment) to the research facility. All corn and complete feed samples were submitted for mycotoxin analysis (Activation Laboratories, Tonawanda, NY).

During the experiment, pens of pigs were weighed, and feed disappearance was recorded every 7 d to determine ADG, ADFI, and G:F. Pigs that died or were removed during this study from the inability to overcome sickness or injury were recorded. Removals were defined as any pig taken from a test pen and placed into a hospital pen where they remained for the duration of the study. Any pig that died while in a test pen or a pig that died from a hospital pen was defined at a mortality.

Blood Sampling

One gilt, closest to the average weight of pigs in that pen (80 total), was bled at the end of the study for immunological, hematological, and biochemical analysis. Blood was collected in tubes without anticoagulant to obtain serum for chemistry and oxidative stress criteria. Blood was allowed to clot before centrifuging for 15 min at 1,500 × g to collect serum, and samples were stored at -80^{o}C until analyzed. Serum samples were sent to Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for antibody titers of porcine circovirus type 2 (PCV2) using an ELISA kit. Serum samples were also sent to Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS) for serum chemistry analysis. Thiobarbituric acid reactive substances (TBARS) were evaluated on serum samples at Kansas State University swine laboratory (Manhattan, KS). Thiobarbituric acid reactive substances assay was a

modification of the methods of Yagi (1998) and Aguilar Diaz De Leon and Borges (2020) and samples were run in triplicate in 96-well microplates.

Blood samples were also collected in tubes containing EDTA to obtain whole blood for hematological analysis. Whole blood was sent to Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS) for complete blood cell counting (CBC). Due to the long transport time from Ohio to Kansas, approximately half of the samples were clotted before analysis could be conducted. As a result, manual leukocyte differentials (Kansas State University Veterinary Diagnostic Laboratory, KS) were performed by a trained veterinary clinical pathologist on the samples that clotted during transportation. Additionally, dried blood spots (DBS) were prepared by placing one drop of blood from each whole blood sample onto a protein saver card (Whatman 903; Cytiva, Marlborough, MA). The DBS cards were sent to University of Ghent, Belgium for mycotoxin analysis. Complete quantification (ng/mL) or mean peak area ± standard deviation of 36 mycotoxins and their phase I and phase II metabolites was simultaneously performed after extraction and LC-MS/MS analysis of spotted blood volume (approximately 60 uL) on Whatman 903 protein saver cards.

Statistical Analysis

Data were analyzed as a randomized complete block design for one-way ANOVA using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Feeder (2 pens of pigs) was considered the experimental unit. Initial pen average BW, sow farm origin, and date of entry into the facility were used as blocking factors. Treatment was used as the fixed effect. For TBARS assay, microplate was used as a random effect. A $\log_2$ transformation was used for PCV2 titers. Studentized residuals were used to evaluate model

assumptions which were reasonably met. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Analysis of DON in the low DON corn was 1.12 ± 0.623 mg/kg (mean ± standard deviation). Analysis of DON in the high DON corn was 2.34 ± 1.809 mg/kg. Deoxynivalenol concentrations in low DON diets were lower compared to high DON diets with the other treatment diets intermediate (Table 2). Inclusion of SMB to high DON diets resulted in lower DON concentrations compared to Technology1 and 1+. Zearalenone and fumonisin were also detected at low levels in treatment diets.

From d 0 to 35, pigs fed the high DON diet had decreased ($P < 0.05$) ADG, ADFI, and final BW compared to pigs fed the low DON diet (Table 3). Pigs fed the high DON+SMB diet had greater ($P < 0.05$) ADG compared to pigs fed the other treatments. Additionally, pigs fed high DON+Technology1+ had increased ($P < 0.05$) final BW compared to the high DON and similar to pigs fed low DON diets. An improvement ($P < 0.05$) in feed efficiency was observed in pigs fed high DON+SMB or high DON+Technology1+ diets compared to pigs fed the low DON or high DON+Technology1 diets with high DON diets intermediate. The differences in ADG and ADFI compared to the high DON diet were mainly driven by the responses in the first 3 wk of the study (Figure 1 and 2). The magnitude of difference was greatest for SMB, then low DON, followed by Technology1+ and 1 in comparison to high DON early in the study. The differences between treatments lessened after wk 3 for both ADG and ADFI.

No differences ($P > 0.10$) were observed for pig removals. Pigs fed high DON+SMB or high DON+Technology1 had lower ($P < 0.05$) mortality and total removals plus mortality compared to pigs fed low DON diets with high DON or high DON+Technology1+ intermediate.

For blood measurements, no differences ($P > 0.10$) were observed between treatments for complete blood count or leukocyte differential analyses (Table 4). In the serum chemistry analysis, pigs fed the high DON+Technology1 diet had a greater ($P < 0.05$) chloride concentration compared to high DON pigs with others being intermediate (Table 5). Additionally, pigs fed high DON had greater ($P < 0.05$) bicarbonate concentration compared to pigs fed high DON+SMB with others being intermediate. There was a statistical difference ($P = 0.038$) in anion gap between treatments, but using a Tukey multiple comparison adjustment no significant pairwise differences were observed. No statistical differences ($P > 0.10$) were observed for PCV2 titers or TBARS between treatments.

For the dried blood spot card, pigs fed high DON or high DON+Technology1 had increased ($P < 0.05$) DON concentrations compared to low DON with high DON+SMB and high DON+Technology1+ intermediate (Table 6). A marginally significant difference ($P = 0.095$) in the number of Beta-zearalenol positive samples was observed with pigs fed high DON diets having the greatest percentage compared to all other treatments. While not statistically different, presence and concentrations of fumonisin B1 and B2, beauvericin, and beta-zearalenol were numerically decreased in pigs fed high DON+Technology1+ with values similar to pigs fed low DON diets.

Discussion

Deoxynivalenol is one of the most common mycotoxins found in cereal grains such as corn, wheat, and barley (Yazar and Omurtag, 2008). This mycotoxin is highly water soluble and is effectively absorbed in the upper gastrointestinal tract (Dänicke et al., 2004; Goyarts et al., 2006; Broekaert et al., 2017). It has been documented that DON ingestion results in reduced feed intake and body weight gain with pigs being the most susceptible livestock species, especially

young pigs (Larivière et al., 2021). A meta-analysis conducted by Andretta et al. (2012) indicated a reduction of 0.28% in body weight gain for each mg/kg of DON in the diet. The data reported herein showed a greater impact from DON as we observed a reduction in body weight gain of 3.6% for each mg/kg of DON in the diet. Furthermore, Patience et al. (2014) observed that pigs fed diets with 4 mg/kg DON had a 6% reduction of ADG and ADFI in comparison to pigs fed diets with less than 1.5 mg/kg DON, which calculates to 2.4% reduction in ADG for each mg/kg of DON in the diet. In our study, we observed a 4% and 6% reduction in ADG and ADFI, respectively when pigs were fed diets with an average of 2.34 mg/kg compared to pigs fed 1.12 mg/kg, with a large portion of the reduction driven by the observations early in the study. Similarly, Shawk et al. (2019) also observed a larger reduction in body weight gain early in the experiment. Although DON effects are primarily driven by decreased feed intake, feed efficiency was also decreased during our experiment. Similar to Frobose et al. (2015), we observed differences in feed efficiency in diets naturally contaminated with DON. The reduction in feed efficiency may be due to pigs wasting feed by sorting through the feed creating wastage.

Although not feeding mycotoxin contaminated grains is the ideal way to reduce the harmful effects of mycotoxins, the use of contaminated feed may be unavoidable. As a result, feed additives with mycotoxin mitigation properties can be used to reduce the toxic effects within the animal. The present study evaluated 3 mitigation strategies (SMB, Technology1 and Technology1+) thought to reduce the negative effects of DON on pig performance. Utilizing feed additives in mycotoxin contaminated grains has been well documented (Frobose et al., 2015; Shawk et al., 2019; Larivière et al., 2021). To the best of our knowledge there is currently no research available that documents the effects of Technology1 or 1+ added to mycotoxin contaminated pig diets.

In diets naturally contaminated with DON, the biological mechanism of SMB is suggested to be due to the chemical alteration of DON to a nontoxic DON-sulfonate adduct form (Young et al., 1987; Frobose et al., 2015). We observed evidence of the potential bioconversion of DON by SMB with diets containing SMB having lower DON concentrations. Previous research indicated improvements in energy and protein utilization in broilers fed sorghum-based diets that were steam pelleted with SMB (Selle et al., 2013, 2014; Truong et al., 2016). The positive effect of SMB on growth is suggested to be due to the oxidative-reductive depolymerization of starch polysaccharides and the reduction of disulfide cross-linkages in proteins resulting in improved starch and protein availability (Truong et al., 2016). Furthermore, SMB has the ability to reduce trypsin inhibitors in soybean meal by the reduction of disulfide cross-linkages (Sessa and Ghantous, 1987; Wang et al., 2009). Therefore, the improved ADG and feed efficiency observed in our study could be explained by the biological effects of SMB or chemical alteration of DON by SMB.

Technology1 and 1+ contain a variety of ingredients including clays, yeast cell wall components and a blend of plant extracts. Yeast cell wall is composed primarily of polysaccharides including beta-glucans which can give adsorptive capacities for mycotoxins through hydrogen and ionic bonding (Jouany et al., 2005). In vitro, beta-glucan has been shown to adsorb aflatoxin B1, DON, and ochratoxin A (Weaver et al., 2014). Furthermore, Technology1+ contains an additional agent of SMB. In our study, when pigs were fed Technology1+, we observed heavier final body weights and improved gain compared to pigs fed high DON or Technology1 diets. This suggests the improved performance observed with Technology1+ could be associated to the addition of SMB. Frobose et al. (2015) also observed heavier final body weights and improved gain when utilizing a feed additive containing SMB.

To further understand how mycotoxins, specifically DON, impact the pig, our study evaluated the effects on immunity, oxidative stress, and organ health. An indicator of immune system function is complete blood count including neutrophils, monocytes, lymphocytes, and eosinophils. Pinton et al. (2008) observed that monocyte numbers can increase in pigs fed 2.2 to 2.5 mg/kg DON. However, in our experiment, these immune criteria were not altered when high concentrations of DON were ingested. Additionally, while not statistically significant, the neutrophil:lymphocyte ratio suggest that other important metabolic processes may be influenced when Technology1 and 1+ are utilized in high DON diets.

Malondialdehyde was used as an indicator of lipid peroxidation (oxidative damage) and when measured in blood serum, we observed lipid peroxidation was not impacted by consumption of high DON diets. However, Chaytor et al. (2011) observed that the combination of 0.182 mg/kg aflatoxin B1 and 0.768 mg/kg DON caused lipid peroxidation. Few studies have determined the ability of DON to cause oxidative stress, especially in pigs (Weaver et al., 2014).

Another important measure evaluated in this study was the effect of DON on organ health, specifically the enzymes gamma glutamyltransferase (GGT) and creatine kinase (CK). Although not statistically significant, pigs fed Technology1+ had numerically reduced GGT and higher levels of CK. Gamma glutamyltransferase plays a significant role in helping the liver metabolize drugs and other toxins (Harvey et al., 1989). Increased CK release into the blood indicates muscle damage potentially associated with the metabolic processes of Technology1+ in the pig warranting further investigation (Hwang et al., 1978; Harvey et al., 1989).

Naturally, pigs have a wide range of normal levels of biological measures (Bollen et al., 2010). Although chloride, bicarbonate, and anion gap levels were statistically different between treatments, the values were not outside of the suggested reasonable range and were similar to

values observed by Weaver et al. (2014). To the best of our knowledge, there is no research available reporting the effect of high DON, SMB, or other DON-detoxifying agents on bicarbonate and anion gap levels in pigs and warrants further investigation.

The combination of several mycotoxins in naturally contaminated corn can result in additive and synergistic effects. Therefore, it is important to analyze multiple mycotoxins through a minimally invasive technique such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) utilized through dried blood spot (DBS) sampling. There has been recent interest in the use of DBS cards to analyze mycotoxins because of the ease of collection and the low blood volume needed, which improves its use even in small animals. Dried blood spot collection only requires one drop of blood placed on a Whatman 903 protein saver card. When utilizing DBS cards in our study, we observed reductions in the presence and concentration of DON, fumonisin B1 and B2, beta-zearalenone, and beauvericin when Technology1+ was added to high DON diets, indicating that other metabolic processes may be influenced when mycotoxin detoxifying feed additives including yeast cell wall components are used. As indicated by Lauwers et al. (2019), DBS cards have the ability to detect 23 different mycotoxins and metabolites. The DBS cards were able to further detect multiple mycotoxins including beta-zearalenol and beauvericin, compared to the mycotoxin analysis utilized for the feed samples. Beauvericin is an ermerging fusarium mycotoxin; however, the relevance and toxicity in pigs is unknown.

In summary, results of this experiment indicate that pigs fed high DON diets had reduced growth performance compared to pigs fed low DON diets. These results also indicated that SMB inclusion in high DON diets provided a benefit in growth performance with ADG and G:F

exceeding growth performance in the low DON diet. The metabolic changes observed in our study by pigs fed high DON+Technology1+ warrant further investigation.

References

Aguilar Diaz De Leon, J., Borges, C. R. 2020. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J. Vis. Exp. (159), e61122, doi:10.3791/61122.

Andretta, I., M. Kipper, C. R. Lehnen, L. Hauschild, M. M. Vale, and P. A. Lovatto. 2012. Meta-analytical study of productive and nutritional interactions of mycotoxins in growing pigs. Animal 6:1476-1482. doi:10.1017/S1751731111002278

Bollen, P. J. A., A. K. Hansen, and A. K. O. Alstrup. 2010. The Laboratory Swine (2nd ed.). CRC Press. doi:10.1201/9781439815304

Broekaert, N., M. Devreese, T. van Bergen, S. Schauvliege, M. De Boevre, S. De Saeger, L. Vanhaecke, F. Berthiller, H. Michlmayr, and A. Malachová. 2017. In vivo contribution of deoxynivalenol-3-β-D-glucoside to deoxynivalenol exposure in broiler chickens and pigs: oral bioavailability, hydrolysis and toxicokinetics. Arch. Toxicol. 91:699-712. doi:10.1007/s00204-016-1710-2

Chaytor, A. C., M. T. See, J. A. Hansen, A. L. P. de Souza, T. F. Middleton, and S. W. Kim. 2011. Effects of chronic exposure of diets with reduced concentrations of aflatoxin and deoxynivalenol on growth and immune status of pigs. J. Anim. Sci. 89:124-135. doi:10.2527/jas.2010-3005

Cheng, Y-H., C-F. Weng, B-J. Chen, and M-H. Chang. 2006. Toxicity of different Fusarium mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pig. Anim. Res. 55:579-590. doi:10.1051/animres:2006032

Dänicke, S., H. Valenta, S. Döll, M. Ganter, and G. Flachowsky. 2004. On the effectiveness of a detoxifying agent in preventing fusario-toxicosis in fattening pigs. Anim. Feed Sci. Technol. 114:141-157. doi:10.1007/s00253-011-3401-5

Dänicke, S., M. Gareis, and J. Bauer. 2001. Orientation values for critical concentrations of deoxynivalenol and zearalenone in diets for pigs, ruminants, and gallinaceous poultry, In:Proc. 10th Soc. Nutr. Physiol., Frankfurt, Germany. p. 171-174.

Ehrlich, K., and K. W. Daigle. 1987. Protein synthesis inhibition by 8-oxo-12,13-epoxytrichothecenes. Biochimica and Biophysica Acta 923:206–213.

Eriksen, G. S., and H. Pettersson. 2004. Toxicological evaluation of trichothecenes in animal feed. Anim. Feed Sci. Technol. 114:205-239. doi:10.1016/j.anifeedsci.2003.08.008

Forsyth, D. M., Y. Yoshizawa, N. Morooka, and J. Tuite. 1977. Emetic and feed refusal activity of deoxynivalenol in swine. Appl. Environ. Microbiol. 34:547-552. doi:10.1128/aem.34.5.547-552.1977

Frobose, H. L., E. D. Fruge, M. D. Tokach, E. L. Hansen, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, and J. L. Nelssen. 2015. The effects of deoxynivalenol-contaminated corn dried distillers grains with solubles in nursery pig diets and potential for mitigation by commercially available feed additives. J. Anim. Sci. 93:1074-1088. doi:10.2527/jas.2013-6883

Goyarts, T., N. Grove, and S. Dänicke. 2006. Effects of the fusarium toxin deoxynivalenol from naturally contaminated wheat five subchronically or as one single dose on the in vivo protein synthesis of peripheral blood lymphocytes and plasma proteins in the pig. Food Chem. Toxicol. 44:1953-1965. doi:10.106/j.fct.2006.06.017

Harvey, R. B., L. F. Kubena, W. E. Huff, D. E. Corrier, D. E. Clark, and T. D. Phillips. 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. Am. J. Vet. Res. 50:602-607.

Hwang P. T., C. J. McGrath, P. B. Addis, W. E. Rempel, E. W. Thompson, and A. Antonik. 1978. Blood Creatine Kinase as a Predictor of the Porcine Stress Syndrome. J. Anim. Sci. 47:630–633. doi:10.2527/jas1978.473630x

Jouany, J. P., A. Yiannikouris, and G. Bertin. 2005. The chemical bonds between mycotoxins and cell wall components of *Saccharomyces cerevisiae* have been identified. *Archiva Zootechnica.* 8:26-50.

Larivière, E. L., C. Zhu, S. Zettell, R. Pattersson, N. A. Karrow, and L. Huber. 2021. The effect of deoxynivalenol-contaminated corn and an immune-modulating feed additive on growth performance and immune response of nursery pigs fed corn- and soybean meal-based diets. Transl. Anim. Sci. 141. doi:10.1093/tas/txab141

Lauwers, M. S. Croubels, S. De Baere, M. Sevastiyanova, E. M. R. Sierra, B. Letor, C. Gougoulias, and M. Devreese. 2019. Assessment of dried blood spots for multi-mycotoxin biomarker analysis in pigs and broiler chickens. Toxins. 11:541. doi:10.3390/toxins11090541

NRC. 2012. Nutrient requirements of swine: 11th revised edition. Natl. Acad. Press, Washington, DC.

Patience, J. F., A. J. Meyers, S. Ensley, B. M. Jacobs, and D. Madson. 2014. Evaluation of two mycotoxin mitigation strategies in grow-finish swine diets containing corn dried distillers grains with solubles naturally contaminated with deoxynivalenol. J. Anim. Sci. 92:620-626. doi:10.2527/jas2013-6238

Pestka, J. J., and A. T. Smolinski. 2005. Deoxynivalenol: toxicology and potential effects on humans. J. Toxicol. Environ. Health B. Crit. Rev. 8:39-69. doi:10.1080/10937400590889458

Pinton, P., F. Accensi, E. Beauchamp, A. Cossalter, P. Callu, F. Grosjean, and I. P. Oswald. 2008. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol. Lett. 177:215-222. doi:10.1016/j.toxlet.2008.01.015

Rotter, B. A., D. B. Prelusky, and J. J. Pestka. 1996. Toxicology od deoxynivalenol (Vomitoxin). J. Toxicol. Environ. Health 48:1-3. doi:10.1080/713851046

Selle, P. H., S. Y. Liu, J. Cai, R. A. Caldwell, and A. J. Cowieson. 2013. Preliminary assessment of including a reducing agent (sodium metabisulphite) in 'all-sorghum' diets for broiler chickens. Anim. Feed Sci. Technol. 186:81-90. doi:10.1016/j.anifeedsci.2013.09.004

Selle, P. H., S. Y. Liu, J. Cai, R. A. Caldwell, and A. J. Cowieson. 2014. Graded inclusions of sodium metabisulphite in sorghum-based diets: I. Reduction of disulphide cross-linkages in vitro and enhancement of energy utilization and feed conversion efficiency in broiler chickens. Anim. Feed Sci. Technol. 190:59-67. doi:10.1016/j.anifeedsci.2013.12.015

Sessa, D. J., and P. E. Ghantous. 1987. Chemical inactivation of soybean trypsin inhibitors. J. Am. Oil Chem. Soc. 64:1682-1687. doi:10.1007/BF02542503

Shawk, D. J., S. S. Dritz, R. D. Goodband, M. D. Tokach, J. C. Woodworth, and J. M. DeRouchey. 2019. Effects of sodium metabisulfite additives on nursery pig growth. Transl. Anim. Sci. 3:103-112. doi:10.1093/tas/txy098

Truong, H. H., D. J. Cadogan, S. Y. Liu, and P. H. Selle. 2016. Addition of sodium metabisulfite and microbial phytase, individually and in combination, to a sorghum-based diet for

broiler chickens from 7 to 28 days post-hatch. Anim. Prod. Sci. 56:1484-1491. doi:10.1071/AN14841

Wang, H., R. J. Faris, T. Wang, M. E. Spurlock, and N. Gabler. 2009. Increased invitro and in vivo digestibility of soy proteins by chemical modification of disulfide bonds. J. Am. Oil Chem. Soc. 86:1093. doi:10.1007/s11746-009-1449-5

Weaver, A. C., M. T. See, and S. W. Kim. 2014. Protective effect of two yeast based feed additives on pigs chronically exposed to deoxynivalenol and zearalenone. Toxins. 6:3336-3353. doi:10.3390/toxins6123336

Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol. 1998. 108:101-6. doi:10.1385/0-89603-472-0:101.

Yazar, S., and G. Z. Omurtag. 2008. Fumonisins, trichothecenes and zearalenone in cereals. Int. J. Mol. Sci. 9:2061-2090. doi:10.3390/ijms9112062

Young, J. C., H. L. Trenholm, D. W. Friend, and D. B. Prelusky. 1987. Detoxification of deoxynivalenol with sodium bisulfite and evaluation of the effects when pure mycotoxin or contaminated corn was treated and given to pigs. J. Agric. Food Chem. 35:259-261. doi:10.1021/jf00074a023

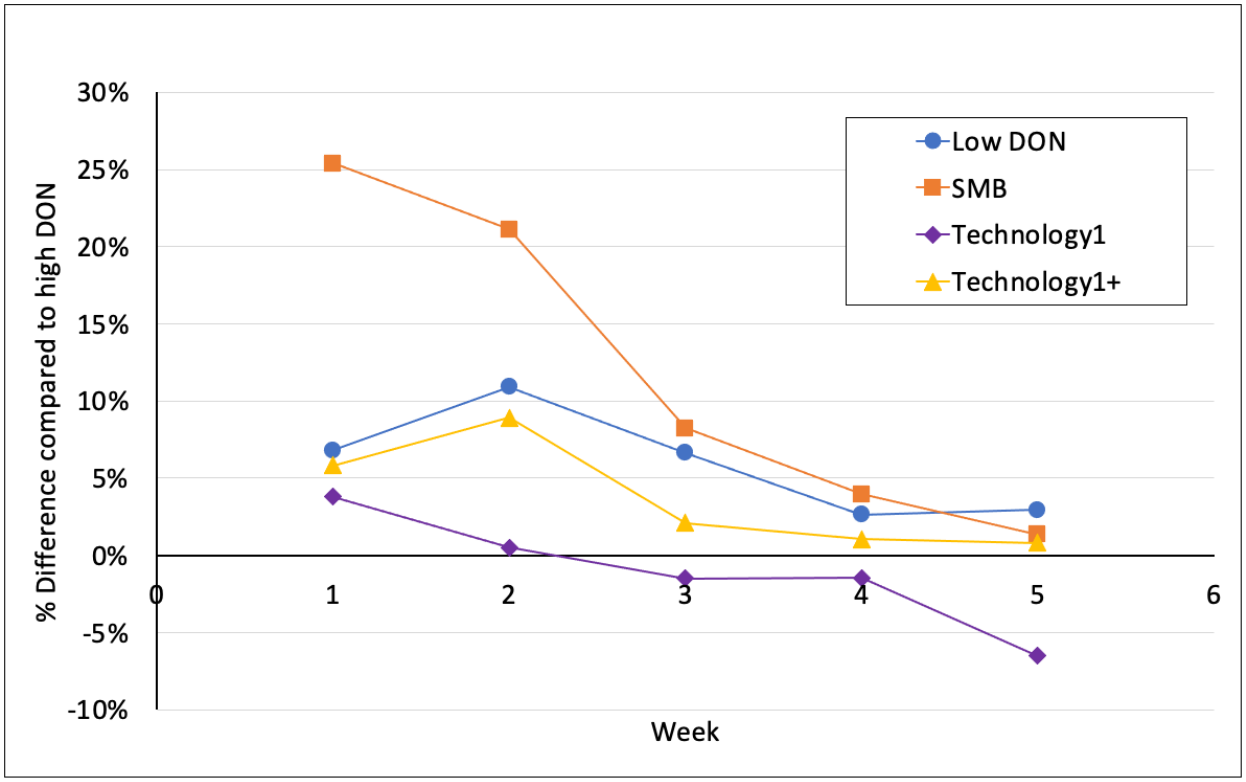

Figure 3.1 Average daily gain by week relative to high DON treatment (horizontal axis). Three feed additives were tested in the high DON diet: sodium metabisulfite (SMB) inclusion rate of 0.50%, Technology1 (Innovad Global; Essen, Belgium) at 0.30%, and Technology1+ (Innovad Global; Essen, Belgium) at 0.30%.

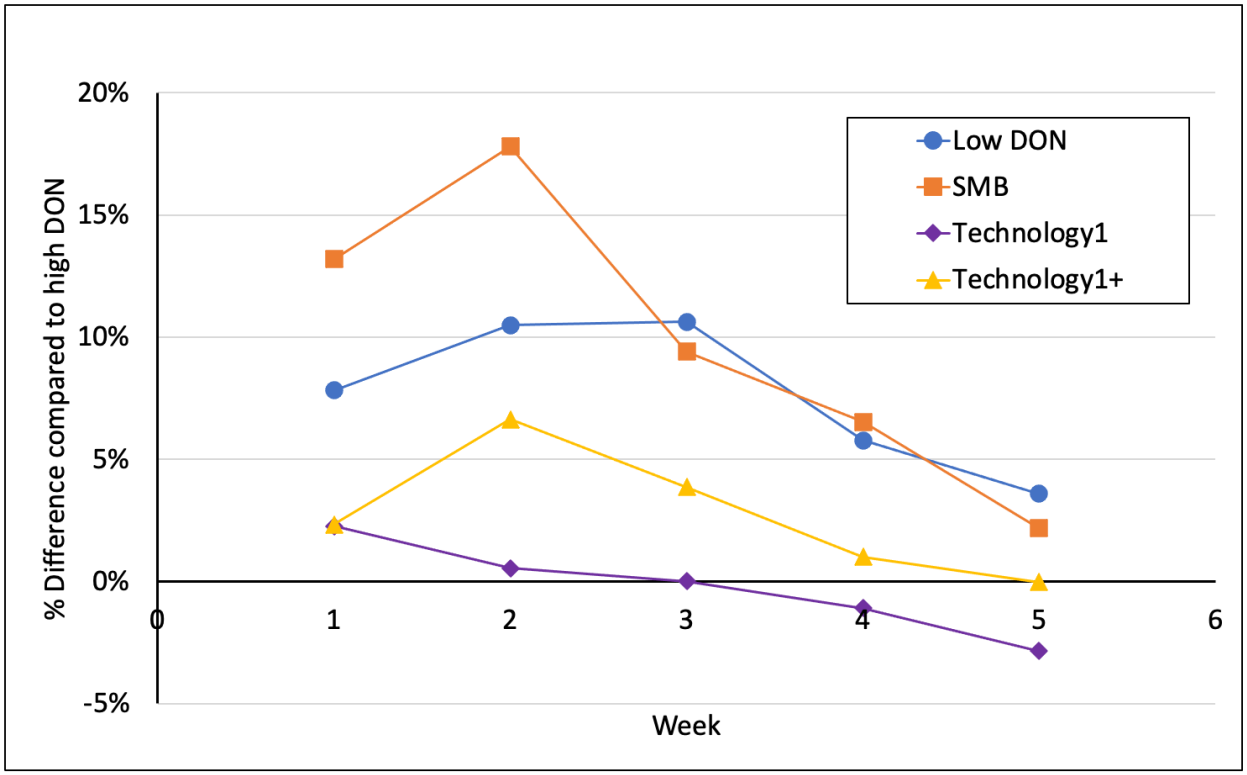

Figure 3.2 Average daily feed intake by week relative to high DON treatment (horizontal axis). Three feed additives were tested in the high DON diet: sodium metabisulfite (SMB) inclusion rate of 0.50%, Technology1 (Innovad Global; Essen, Belgium) at 0.30%, and Technology1+ (Innovad Global; Essen, Belgium) at 0.30%.

Table 3.1. Diet composition (as-fed basis)[1]

Ingredient, %	6 to 25 kg BW diet[2]
Corn	62.63
Soybean meal, dehulled	22.02
Soybean meal, expelled	10.75
Limestone	1.10
Monocalcium P	1.20
Salt	0.73
L-Lys-HCl	0.48
DL-Met	0.25
L-Trp	0.05
L-Val	0.15
L-Thr	0.32
Zinc oxide	0.01
Copper sulfate	0.05
Vitamin and trace mineral premix	0.18
Phytase[3]	0.10
Feed additive[4]	---
Total	100
Calculated analysis[5]	
Lys	1.35
Ile:Lys	56
Leu:Lys	114
Met:Lys	39
Met and Cys:Lys	60
Thr:Lys	65
Trp:Lys	20.4
Val:Lys	71
His:Lys	37
Total Lys, %	1.49
Net energy, kcal/kg	2,429
SID Lys:NE, g/Mcal	5.56
CP, %	21.4
Ca, %	0.77
STTD P, %	0.52

[1]A common starter pellet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study.

[2]Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.

[3]Quantum Blue 2G (AB Vista, Marlborough, UK) provided an estimated release of 0.14% STTD P.

[4]Three feed additives were tested in the high DON diet: sodium metabisulfite (SMB) inclusion rate of 0.50%, Technology1 (Innovad Global; Essen, Belgium) at 0.30%, and Technology1+ (Innovad Global; Essen, Belgium) at 0.30%. In each diet, additives were included at the expense of corn.

[5]NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 3.2 Mycotoxin analysis of corn and complete treatment diets

Item, mg/kg	Low DON	High DON	High DON + SMB[1]	High DON + Technology 1[2]	High DON + Technology 1+[3]
Corn[4]					
DON	2.46	4.17	---	---	---
Zearalenone	0.08	0.10	---	---	---
Fumonisin	0.15	ND[6]	---	---	---
Complete treatment diets[5]					
DON	1.12	2.34	1.44	2.20	1.66
Zearalenone	0.03	0.13	0.09	0.06	0.16
Fumonisin	0.04	0.08	0.06	0.10	0.08

[1]Sodium metabisulfite (SMB) inclusion rate of 0.50%.

[2]Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[3]Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[4]Low and high DON corn samples were collected during the study including 1 set of samples at the beginning of the study and 1 set of samples at the conclusion of the study. Values in the table represent the average mycotoxin concentration throughout the study.

[5]Feed samples were collected from at least 6 feeders per treatment per feed delivery (approximately 4 to 5 deliveries depending on the treatment) to the research facility and submitted to Activation Laboratories (Tonawanda, NY) for mycotoxin analysis. Values in the table represent the average mycotoxin concentration throughout the study.

[6]ND = samples did not contain detectable mycotoxin.

Table 3.3 Evaluation of mycotoxin control strategies on nursery pig growth performance[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
BW, kg							
d 0	6.5	6.5	6.5	6.5	6.5	0.08	0.432
d 35	25.3[a,b]	24.2[c]	25.7[a]	23.8[c]	24.8[b]	0.23	< 0.001
d 0 to 35							
ADG, g	522[b]	499[c,d]	542[a]	489[d]	512[b,c]	6.8	< 0.001
ADFI, g	723[a]	680[b]	734[a]	675[b]	693[b]	8.8	< 0.001
G:F, g/kg	722[b]	733[a,b]	738[a]	724[b]	738[a]	3.8	0.001
Removals, %	1.27	1.08	1.17	0.98	0.88	0.415	0.923
Mortality, %	4.47[a]	2.42[a,b]	1.91[b]	2.08[b]	3.43[a,b]	1.203	0.002
Total removals and mortality, %	6.14[a]	3.69[a,b]	3.20[b]	3.22[b]	4.62[a,b]	1.368	0.007

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study.

[2] Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.

[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.

[4] Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[5] Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.

Table 3.4 Evaluation of mycotoxin control strategies on nursery pig blood criteria[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
Complete blood count[6]							
Sample count, *n*	5	6	6	9	5	---	---
Erythrocyte concentration, M/uL	5.73	5.77	6.08	5.75	5.89	0.190	0.592
Hemoglobin, g/dL	10.87	10.93	11.53	11.18	11.08	0.346	0.585
Cellular hemoglobin, g/dL	10.09	10.28	10.51	10.26	10.24	0.326	0.901
Hematocrit spun, %	34.68	35.35	37.38	35.12	35.55	1.254	0.483
Mean cell volume, fL	64.62	64.80	64.17	64.44	63.34	1.861	0.982
Mean cell hemoglobin, pg	19.14	19.03	19.02	19.47	18.78	0.526	0.862
Mean cell hemoglobin concentration, g/dL	29.64	29.38	29.68	30.24	29.64	0.292	0.152
Cell hemoglobin concentration mean g/dL	27.46	27.57	27.07	27.77	27.46	0.338	0.541
RBC distribution width, %	18.19	17.35	17.82	17.84	18.47	0.650	0.663
Leukocyte count, K/uL	20.14	20.02	19.65	19.68	19.34	1.882	0.998
Segmented neutrophil concentration, K/uL	7.51	7.53	7.65	6.06	5.78	1.273	0.496
Total neutrophils, K/uL	7.51	7.53	7.65	6.06	5.78	1.273	0.496
Lymphocyte concentration, K/uL	10.08	11.45	10.92	12.79	12.48	1.433	0.566
Monocyte concentration, K/uL	0.66	1.16	1.42	0.86	1.10	0.326	0.432
Eosinophil concentration, K/uL	0.12	0.12	0.10	0.15	0.14	0.073	0.967
Plasma protein, g/dL	5.77	5.87	5.90	5.85	5.68	0.191	0.879
Fibrinogen, mg/dL	311.83	285.96	309.47	221.02	254.64	46.297	0.382
Neutrophil:Lymphocyte ratio	0.89	0.68	0.69	0.50	0.50	0.147	0.209
Leukocyte differential[7]							
Sample count, *n*	15	16	16	16	16	---	---
Segmented neutrophil, %	39.05	33.40	34.16	31.97	30.02	2.845	0.211
Band neutrophil, %	0.15	0.00	0.13	0.06	0.19	0.099	0.581
Total neutrophil, %	39.19	33.40	34.29	32.03	30.21	2.871	0.218
Lymphocyte, %	57.73	61.78	59.48	63.92	64.74	3.016	0.369

Monocyte, %	2.43	4.10	5.01	3.51	4.06	0.709	0.120
Eosinophil, %	0.45	0.59	1.07	0.55	0.70	0.236	0.368
Basophil,%	0.08	0.00	0.18	0.07	0.13	0.076	0.491
Neutrophil:Lymphocyte ratio	0.79	0.60	0.66	0.52	0.49	0.092	0.129

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Blood samples were collected from 1 average weight gilt of each experimental unit at the end of the study.

[2] Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.

[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.

[4] Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[5] Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[6]Banded neutrophil concentration was 0 K/uL for all 31 samples. Basophil concentration was non-detectable for 28 of the 31 samples. Count of samples with detectable levels were 1, 0, 2, 0, 0 across treatments low DON, high DON, high DON+SMB, high DON+Technology1, high DON+Technology1+, respectively. Detectable levels were 0.3 K/uL for treatment low DON and 0.2 K/uL for high DON+SMB.

[7]Metamyelocyte and myelocyte were non-detectable for all 79 samples. Nucleated erythrocytes per 100 WBC was non-detectable for 70 of the 79 samples. Count of samples with detectable levels were 2, 3, 2, 0, 2 across treatments low DON, high DON, high DON+SMB, high DON+Technology1, high DON+Technology1+, respectively.

[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.

Table 3.5 Evaluation of mycotoxin control strategies on nursery pig serum criteria[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
Serum chemistry[6]							
Glucose, mg/dL	107.88	105.38	105.25	110.7	109.50	2.365	0.366
Urea nitrogen, mg/dL	8.69	9.44	8.81	9.63	7.88	0.694	0.415
Creatinine, mg/dL	0.59	0.62	0.69	0.67	0.63	0.052	0.717
Protein total, g/dL	4.91	4.86	5.06	4.83	4.92	0.077	0.243
Albumin, g/dL	3.84	3.76	3.86	3.71	3.83	0.075	0.551
Globulin calculated, g/dL	1.07	1.10	1.20	1.12	1.09	0.055	0.515
Calcium total, mg/dL	11.31	10.96	11.10	11.04	11.39	0.131	0.104
Phosphorus, mg/dL	11.73	11.31	11.53	11.35	11.87	0.233	0.289
Sodium, mmol/L	142.75	142.00	142.81	141.75	142.62	0.634	0.531
Potassium, mmol/L	6.41	6.26	6.55	6.59	6.63	0.285	0.880
Sodium:potassium	22.75	23.25	22.38	23.00	22.19	0.937	0.928
Chloride, mmol/L	100.13^{ab}	99.81^{b}	101.00^{ab}	101.25^{a}	100.13^{ab}	0.380	0.007
Bicarbonate, mmol/L	26.63^{ab}	27.69^{a}	24.94^{b}	25.94^{ab}	26.50^{ab}	0.558	0.016
Anion gap calculated, mmol/L	23.56	22.00	24.75	22.05	23.81	0.740	0.038
Aspartate transaminase P5P, U/L	68.00	70.75	56.38	61.53	75.00	7.072	0.345
Alkaline phosphatase, U/L	268.06	275.31	252.44	264.44	275.31	11.559	0.584
Gamma glutamyltransferase, U/L	60.06	70.44	59.56	71.88	57.63	5.538	0.213
Creatine kinase, U/L	2077.3	2559.2	1881.4	2766.8	3338.1	472.89	0.168
Porcine circovirus type 2 titer	9.03	9.42	9.24	8.93	8.58	0.235	0.108
TBARS, µM MDA[7]	13.07	12.30	12.56	12.86	13.66	0.960	0.567

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Serum samples were collected from 1 average weight gilt of each experimental unit at the end of the study.

[2] Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.

[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.

[4] Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[5] Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[6] Sorbitol dehydrogenase (SDH) below detection limit of 0.3 U/L for 73 of 80 samples. Count of samples with detectable SDH levels were 2, 1, 2, 1, 1 for treatments low DON, high DON, high DON+SMB, high DON+Technology1, high DON+Technology1+, respectively. Total and direct bilirubin were below detection limit of 0.2 mg/dL for all 80 samples.

[7]TBARS = Thiobarbituric acid reactive substances. μM of MDA (malondialdehyde) equivalent.

[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.

Table 3.6 Evaluation of mycotoxin control strategies on nursery pig dried blood spot cards[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
DON							
Concentration, ng/mL	7.44[b]	10.00[a]	8.04[a,b]	9.78[a]	9.31[a,b]	0.596	0.006
Positive, %	77.4	100.0	77.4	94.9	100.0	12.42	0.703
Count of samples LOD < result < LOQ, *n*	0	0	0	0	0	---	---
DON glucuronide							
Peak area	1,004	1,330	987	1,363	1,209	154.9	0.156
Positive, %	38.3	78.5	28.7	78.5	59.4	20.07	0.155
Count of samples LOD < result < LOQ, *n*	0	0	0	0	0	---	---
DOM-1							
Concentration, ng/mL	---	3.59	2.82	---	3.58	2.454	0.904
Positive, %	34.8	42.2	64.8	34.8	64.8	14.79	0.339
Count of samples LOD < result < LOQ, *n*	6	6	5	6	8	---	---
Fumonisin B1							
Concentration, ng/mL	4.83	7.52	4.84	5.43	4.67	1.852	0.526
Positive, %	43.2	63.5	56.8	43.2	43.2	13.39	0.719
Count of samples LOD < result < LOQ, *n*	4	4	6	3	3	---	---
Fumonisin B2							
Concentration, ng/mL	---	146.4	146.4	43.4	---	141.90	0.860
Positive, %	9.5	15.2	21.4	15.2	---	11.91	0.926
Count of samples LOD < result < LOQ, *n*	2	1	3	2	0	---	---
Beauvericin							
Peak area	1,782	3,050	2,835	2,346	2,047	649.6	0.410
Positive, %	56.3	56.3	37.5	50.0	56.3	12.55	0.831
Count of samples LOD < result < LOQ, *n*	0	0	0	0	0	---	---
Beta-zearalenol							

Concentration, ng/mL	7.93	6.41	10.32	6.08	7.95	2.10	0.266
Positive, %	59.1	94.1	68.1	83.4	40.4	18.05	0.095
Count of samples LOD < result < LOQ, *n*	3	8	6	7	5	---	---

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Dried blood spots (DBS) were prepared by placing one drop of blood from each whole blood sample onto a protein saver card. DBS cards were sent to University of Ghent, Belgium for mycotoxin analysis. Complete quantification (ng/mL) or mean peak area ± standard deviation of 36 mycotoxins and their phase I and phase II metabolites was simultaneously performed after extraction and LC-MS/MS analysis of spotted blood volume (approximately 60 uL) on Whatman 903 protein saver cards. Concentration is reported by treatment for all samples with a sample result > limit of quantification (LOQ). Positive samples are reported as percentage of samples within treatment with result > limit of detection (LOD). Samples with a result of trace detection of metabolite (LOD < sample result < LOQ) are reported as count by treatment. One sample had trace levels of Fumonisin B3 from the High DON treatment.

[2]Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.

[3]Sodium metabisulfite (SMB) inclusion rate of 0.50%.

[4]Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[5]Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.

[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.