The effects of fumonisin and high protein dried distillers grain on pig growth performance

by

Zhong-Xing Rao

B.S., National Chung Hsing University, Taiwan, 2017

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

2020

Approved by:

Major Professor

Mike Tokach

Copyright

Abstract

Experiment 1 used a total of 350 pigs to determine the effects of increasing fumonisin concentration from 7.2 to 35.1 mg/kg on nursery pig growth performance and serum sphinganine (**SA**) to sphingosine (**SO**) ratio. Experiments 2 and 3 used a total of 650 pigs to determine the efficacy of various commercial products on growth performance and serum SA:SO ratios of nursery pigs fed high fumonisin diets. Experiment 4 used a total of 1,890 pigs to determine the effects of pigs fed diets with high-protein dried distillers grains (**HPDDG**) or conventional dried distillers grains with solubles (**DDGS**) on growth performance and carcass characteristics. Experiment 1 determined that increasing fumonisin concentration linearly reduced growth performance and final BW, and linearly increased serum SA:SO ratios. These results demonstrate that for 20- to 60-lb nursery pigs, diets containing greater than 32.7 mg/kg of fumonisin should be avoided, as increasing fumonisin concentration worsens growth performance and serum SA:SO ratio. In Exp. 2 and 3, growth performance and serum SA:SO ratios were improved in pigs fed high fumonisin diet with Biofix Select Pro, but not with Kallsil Dry or Feed Aid Wide Spectrum. The improvement in serum SA:SO ratios with Biofix Select Pro was only found in pigs fed 30 mg/kg of fumonisin (Exp. 3), but not 60 mg/kg (Exp. 2). In Exp. 4, there were no differences observed in ADG between pigs fed either DDG sources. Increasing either conventional DDGS or HPDDG decreased carcass yield and HCW; however, there were no differences between pigs fed HPDDG or conventional DDGS. Iodine value (IV) increased with increasing either DDG sources, and was greater in pigs fed HPDDG than conventional DDGS, which was probably due to the difference in oil content.

Table of Contents

List of Figures vi
List of Tables vii
Acknowledgements viii
Chapter 1 - Effects of Fumonisin-Contaminated Corn on Growth Performance of 9- to 28-kg Nursery Pigs 1
Abstract 1
Introduction 2
Materials and Methods 4
Mycotoxin analysis 5
Serum SA and SO analysis 6
Statistical analysis 7
Results and Discussion 8
References 13
Chapter 2 - Efficacy of commercial products on nursery pig growth performance fed diets with fumonisin contaminated corn 27
Abstract 27
Introduction 28
Materials and Methods 29
Serum SA and SO analysis 32
Statistical analysis 33
Results 33
Fumonisin analysis 33
Experiment 1 34
Experiment 2 35
Discussion 36
References 43
Chapter 3 - Evaluation of high-protein distillers dried grains on growth performance and carcass characteristics of growing-finishing pigs 60
Abstract 60

Introduction 62
Statistical analysis 66
Results and Discussion 66
References 72

List of Figures

Figure 1-1 Weekly average daily gain of pigs fed increasing fumonisin concentration. 24
Figure 1-2 Weekly average daily feed intake of pigs fed increasing fumonisin concentration.... 25
Figure 1-3 Weekly feed efficiency of pigs fed increasing fumonisin concentration. 26
Figure 2-1. Icteric serum on d 14 of Exp. 1 59

List of Tables

Table 1-1 Diet composition (as-fed basis)[1,2] 18
Table 1-2 Chemical analysis of diets (as-fed basis)[1,2] 20
Table 1-3 Dietary mycotoxin level (as-fed basis)[1, 2, 3] 21
Table 1-4 Effect of fumonisin concentration on 9- to 28-kg nursery pig growth performance [1, 2] 22
Table 1-5 Effect of fumonisin concentration on serum sphinganine (SA)-to- sphingosine (SO) ratio[1,2,3] 23
Table 2-1 Dietary mycotoxin levels, Exp. 1 (as-fed basis, mg/kg)[1, 2] 50
Table 2-2 Dietary mycotoxin levels, Exp. 2 (as-fed basis, mg/kg)[1, 2] 51
Table 2-3 Diet composition (as-fed basis)[1] 52
Table 2-4 Diet composition, Exp. 2 (as-fed basis) 54
Table 2-5 Effect of commercial products on growth performance of nursery pigs, Exp. 1[1] 56
Table 2-6 Effect of commercial products on growth performance of nursery pigs, Exp. 2[1,2,3] 58
Table 3-1 Chemical analysis of ingredients (as-fed basis)[1,2] 76
Table 3-2 Dietary mycotoxin concentrations (as-fed basis, ug/kg)[1] 77
Table 3-3 Composition of phase 1 and 2 diets (as-fed basis)[1, 2] 78
Table 3-4 Composition of phase 3 and 4 diets (as-fed basis)[1, 2] 80
Table 3-5 The effects of DDG source and level on grow-finish pig growth performance, and carcass characteristics[1, 2] 82

Acknowledgements

This thesis was made possible because of the help of all the people who have been in my life. First, I'd like to thank all the professors at National Chung-Hsing University for leading me into the broad world of animal science. Without you guys, I would never have a chance to be here. I'd also like to thank my major professors, Dr. Mike Tokach and Dr. Jason Woodworth for their unreserved mentorship that made me who I am today. Dr. Joel DeRouchey, Dr. Bob Goodband, and Dr. Steve Dritz, thank you all for the knowledge and support that gave me a challenging and enjoyable graduate school experience. I am blessed to work with all five of you.

Furthermore, I'd like to thank all the graduate students who I have encountered in graduate school. You are not just colleagues, but also friends that I will always cherish. You teach me more than any class in this world can do. The office is like a treasure box that you can always be amazed by what you can learn there. You made my time here invaluable.

Lastly, I'd like to thank all the support of my family. You raised and taught me how to be a hardworking and responsible person. The path you paved for me has been the most important resource in my life. It is something that I will never be able to payback.

Chapter 1 - Effects of Fumonisin-Contaminated Corn on Growth Performance of 9- to 28-kg Nursery Pigs

Abstract

Fumonisin contamination in corn is an emerging issue in animal feed production around the world. Fumonisin disrupts the metabolism of sphingolipids, causes damage to animal tissue, and reduces growth performance. This experiment was conducted to determine the effect of feeding fumonisin contaminated corn on growth performance and sphinganine (**SA**) to sphingosine (**SO**) ratios of 9- to 28-kg nursery pigs. A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 8.9 kg) were used. Dietary treatments consisted of fumonisin-contaminated corn (approximately 50 mg/kg of fumonisin B1+B2) blended with low fumonisin corn (approximately 10 mg/kg of fumonisin B1+B2). After blending, the final diets were analyzed to contain fumonisin (B1+B2) concentrations of 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg. After weaning, pigs were fed common diets for 21 days before the experiment started. Then, on day 21 after weaning, considered day 0 of the experiment, pens of pigs were assigned to treatments in a randomized complete block design with initial weight as the blocking factor. Experimental diets were fed in mash form for 28 d. There were 5 pigs per pen and 14 pens per treatment. From d 0 to 28, increasing fumonisin decreased (linear, $P < 0.001$) average daily gain (**ADG**) and final body weight (**BW**), average daily feed intake (**ADFI**; linear, $P = 0.055$), and gain:feed ratio (**G:F:** linear, $P = 0.016$). Although these response criteria tested linear, the greatest reduction in ADG was observed in pigs fed 32.7 and 35.1 mg/kg of fumonisin. Increasing fumonisin increased the serum SA:SO ratio (linear, $P < 0.001$) on d 14 and 28, which corresponded with the decreased growth performance. Data suggest that the serum SA:SO ratio is a reliable biomarker indicating fumonisin intoxication. In summary, for 9- to 28-kg nursery

pigs, diets containing greater than 32.7 mg/kg of fumonisin should be avoided, as increasing fumonisin concentration worsens growth performance and increases serum SA:SO ratio. Furthermore, diets containing greater than 21.9 mg/kg should be evaluated with caution as further research is warranted to determine the effect of fumonisin concentration between 21.9 and 32.7 mg/kg.

Keywords: Fumonisin, Growth, Mycotoxin, Nursery pigs, Sphinganine, Sphingosine

Introduction

Fumonisins are a group of mycotoxins mainly produced by *Fusarium vertcilliodes* and *F. proliferatum*. There are 28 fumonisin homologs, and they are highly polar compounds and soluble in water. Fumonisin B1 is the most prevalent and toxic of the fumonisin family, while fumonisin B2 and B3 are less prevalent and are usually associated with FB1 but at lower concentrations. Fumonisin contamination in corn has been an emerging issue in animal feed production (Hendel et al., 2020). Pigs fed fumonisin-contaminated corn have reduced growth performance, and damage to the liver, lungs (Zomborszky-Kovacs et al., 2002), kidneys (Colvin et al., 1993), and gastrointestinal structure (Bouhet et al., 2004). According to previous research, less than 25 mg/kg of fumonisin causes no apparent clinical changes, but when pigs were fed more than 50 ppm reduced growth performance and liver damage were observed (Ensely and Radke, 2019). High doses (above 100 mg/kg of feed) of fumonisin contamination in swine feed for a short period of time (approximately 3 to 5 days) can cause acute porcine pulmonary edema (**PPE**) which is often lethal (Ensely and Radke, 2019). The U.S. Food and Drug Administration established a guidance level of 20 mg/kg total fumonisin (B1+B2+B3) as a maximum in corn

used for swine feed and at this level of contamination, corn should not exceed 50% of the diet (U.S. Food & Drug Administration, 2001). The European Commission also established a recommendation for fumonisin (B1+B2) levels for corn used in animal feed to be below 60 mg/kg and no more than 5 mg/kg in complete swine diets (European Commission, 2016). Fumonisin has a similar structure to sphinganine (**SA**) and sphingosine (**SO**), therefore it can work as a competitive inhibitor for ceramide synthase, a crucial enzyme for synthesizing complex sphingolipids from SA (via *de novo* synthesis) and SO (via sphingolipid turnover) in animals (Merrill et al., 2001). Sphingolipids are important components for cell membrane and lipid-rich structures. They serve as binding sites for extracellular matrix proteins, modulators for growth factor receptors, and precursors for second messengers of growth factor and other cell responses (Merrill et al., 2001). Disrupted sphingolipid metabolism causes cell damage and apoptosis in organs, such as the liver and kidney (Fodor et al., 2006). Without being converted to sphingolipids by ceramide synthase, SA and SO accumulate in tissue. Sphinganine accumulates at a greater rate than SO, therefore serum SA:SO ratio has been used as a biomarker to determine the severity of fumonisin intoxication (Zomborszky et al., 2000; Hsiao et al., 2007; Dilkin et al., 2010). To our knowledge, there is limited data to determine the concentration of naturally contaminated fumonisin in corn that will affect pig growth performance and serum SA:SO ratio. Because co-occurrence of fumonisin with other mycotoxins in nature are common (Smith et al., 2016), most previous studies have determined the effects of fumonisin on pigs by utilizing purified fumonisin toxin or cultured feed material (Bracarense et al., 2012; Mateos et al., 2018; Schertz et al., 2018), but not naturally contaminated fumonisin corn (Riley et al., 1993; Motelin et al., 1994). Therefore, the objective of this study was to determine the effects of feeding corn

naturally contaminated with fumonisin on growth performance and SA:SO ratio of 9- to 28-kg nursery pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (1.2 × 1.2 m) provided 0.28 m^2 per pig and was equipped with a 4-hole, dry self-feeder, and a nipple waterer to provide *ad libitum* access to feed and water.

A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 8.9 kg) were used in a 28-d growth trial. Pigs were weaned at approximately 21 d of age and placed in pens of 5 pigs each based on initial weight and gender. A common phase 1 pelleted diet was fed for 7 d and a common phase 2 mash diet was fed for another 14 d. At d 21 after weaning, which was considered d 0 of the trial, pens of pigs were randomly allotted to treatment in a randomized complete block design with weight as the blocking factor. There were 14 replicate pens per treatment. Pen weights and feed disappearance were measured weekly to determine ADG, ADFI, and G:F.

All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Two diets were formulated using low fumonisin corn (10 mg/kg of fumonisin B1+B2; control) or fumonisin-contaminated corn (approximately 50 mg/kg fumonisin B1+B2; Table 1). These two diets were blended at the feed mill to produce three additional diets with intermediate fumonisin concentrations. Consequently, five dietary treatments were manufactured with final diets containing 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg of

analyzed fumonisin (B1+B2). All diets were formulated to contain 1.30% standardized ileal digestible Lys and met or exceeded the NRC (2012) nutrient requirement estimates.

Representative diet samples were obtained from every fifth bag of feed manufactured. Samples were analyzed for dry matter (method 935.29; AOAC Int., 2019), crude protein (method 990.03; AOAC Int., 2019) , Ca (method 6.3; Kovar, 2003) , P (method 6.3; Kovar, 2003) , neutral detergent fiber (ANKOM Technology, 2005), and ether extract (ANKOM Technology, 2004; Laboratories Inc., Kearney, NE; Table 2).

Mycotoxin analysis

Representative complete diet samples were sent to North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) for mycotoxin analysis. The multiple mycotoxin assay is based on an Agilent Technologies method for mycotoxin in maize using ultra high pressure liquid chromatography and tandem mass spectrometric detection (**UHPLC/MS/MS**) with modifications (Varga et al., 2013). It was adapted to quantitate for the following mycotoxins: aflatoxins (**AFB1, AFB2, AFG1, AFG2**), fumonisins (FB1, FB2), ochratoxin, deoxynivalenol, zearalenone, T-2 toxin, HT-2, citrinin (screen not quantitative) and, sterigmatocystin. Matrix effects are compensated for by using 13-carbon-labeled (**13C**) mycotoxins as an internal standard for each of the 13 target compounds. The standards and 13C-labeled standards were purchased from Romer Labs (Biopure™, Getzersdorf, Austria). The limits of quantitation (LOQ) for this UHPLC/MS/MS assay at parts per billion (ppb) levels vary with the mycotoxin but are consistent across all matrices, are below concentrations listed in the US Food and Drug Administration (**FDA**) guidelines for mycotoxins in animal feeds, and are at practical concentrations for mycotoxins detected in animal feeds in the US. Twenty-five grams

of ground and homogenized feed sample was extracted in acetronitrile (LC-MS grade)/nanopure water (50/50, v/v) containing 0.1% formic acid. Samples were shaken for 60 min, diluted with extracting solution, centrifuged at 13 °C, and an internal standard was added to final extract. Analysis was carried out on Agilent 6460 Triple Quad LC/MS (Agilent Technologies Inc, Santa Clara, CA) with positive electrospray ionization in Dynamic MRM (multiple reaction monitoring) mode using two major transitions per target compound and one transition for the (13 °C) labeled internal standards.

Serum SA and SO analysis

Serum samples were collected on d 0, 14, and 28 for the determination of serum SA:SO ratio. Blood samples were collected from two pigs per treatment and analyzed as a baseline concentration for all treatments on d 0. Blood samples were collected from nine pigs per treatment on d 14 and 28. For each selected pen, the median weight pig was selected. The same pig per pen was bled for the d 14 and 28 collections. Whole blood samples were allowed to clot for 30 min, centrifuged at 1,500 × g for 15 min. and the resulting serum supernatants were transferred to polypropylene tubes and stored at -80°C. Serum samples were sent to the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) and analyzed for sphinganine/sphingosine by HPLC with fluorescence detection utilizing a modification of the method of Hsiao et al (2007) and Riley et al. (1994). Individual sphinganine and sphingosine concentration vary between individuals, but Sa:So ratios are relatively stable and have been proposed to be an indicator for fumonisin toxicosis. Briefly, 0.5 mL of serum sample was transferred to 15 mL polypropylene tubes and 2.0 mL of methanolic 0.125M KOH and 500 uL of chloroform was added. The samples were vortexed and incubated in a water bath at 37°C for a

minimum of 30 min. Sphinganine and sphingosine were extracted by adding 2.0 mL chloroform, 2.0 mL alkaline water, and 500 µL of 2N ammonium hydroxide. The samples were vortexed, centrifuged at 224 × g for 10 min and 2 mL of the lower chloroform layer was transferred to polypropylene vials containing 4 mL alkaline water. The vials were vortexed, centrifuged for 10 min at 224 × g and the upper phases were discarded, and the lower chloroform layers were evaporated to dryness. The residues were reconstituted in 600 uL of 80% methanol and transferred to autosampler vials. OPA reagent (300 uL) was added and the derivatized samples were analyzed along with appropriate standards by HPLC. The HPLC system consisted of a Hitachi Model L-7100 pump, Hitachi Model L-7485 fluorescence detector (ex-230 nm; em-430 nm), Hitachi Model L-7200 autosampler with Hitachi D-7000 data acquisition interface and ConcertChrom software. The HPLC column was a 250 × 4.6 Synergi 4um Polar RP (Phenomenex) with a C18 SecurityGuard precolumn (Phenomenex) and a mobile phase of methanol: 0.005 M K2HPO4 buffer (pH7.0) (850:150) at a flow rate of 0.8 mL/min.

Statistical analysis

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. For every response, 2 analytical models were constructed by using homogenous variance and heterogenous variance models weighted by treatment. After comparing the 2 models, one was selected based on the ANOVA test ($P \leq 0.05$) via Bayesian information criterion. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing fumonisin on ADG, ADFI, G:F, BW, and serum SA:SO ratio. Contrast coefficients were adjusted for unequally spaced treatments. Interactive effects of fumonisin level and growth period (week) was tested as repeated measurement. Data were analyzed using the R

program (R Core Team, 2019). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

The corn used in this study had high naturally contaminated fumonisin concentrations without detectable levels of other mycotoxins (Table 3). This allowed us to exclude the interactive effects of multiple mycotoxins on growth performance and serum SA:SO ratio. The dietary fumonisin (B1+B2) levels were 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg. The ratios of fumonisin B1 to B2 ranged from 3.4 to 4.0:1, which were approximate to the previously reported 3:1 ratio (Wilson, 2012). Fumonisin B3 was not analyzed due to its much lower toxicity compared to fumonisin B1 and B2 (Ensely and Radke, 2019). The diet with 7.2 mg/kg of fumonisin (B1+B2) was manufactured with corn with the lowest mycotoxin contamination that could be obtained locally in the same crop year, which suggested that this corn still contained about 11 mg/kg of fumonisin (B1+B2). Corn with 11 mg/kg of fumonisin may not pose immediate danger toward swine but would endanger equines due to their higher sensitivity toward fumonisin (Ensely and Radke, 2019). The highest fumonisin diet (35.1 mg/kg of B1+B2) suggests that the high fumonisin-contaminated corn contained about 50 mg/kg of fumonisin (B1+B2). This level would cause negative effects to pigs fed common corn-soybean meal diets according to previous researchers (Ensely and Radke, 2019).

Increasing dietary fumonisin (B1+B2) concentration from 7.2 to 35.1 mg/kg for 28 d decreased (linear, $P < 0.001$) overall ADG and d 28 BW, overall ADFI (linear, $P = 0.055$), and overall G:F (linear, $P = 0.016$; Table 4). Serum SA:SO ratios on d 14 and 28 were linearly increased ($P < 0.001$) as dietary fumonisin (B1+B2) concentration increased (Table 5). Although

testing linear, the greatest reduction in overall growth performance and increase in serum SA:SO ratios were observed in pigs fed 32.7 and 35.1 mg/kg of fumonisin (B1+B2). There were no apparent external clinical signs of fumonisin intoxication, such as coughing, except reduced growth performance and increased serum SA:SO ratio. There was no evidence of difference on percentage of pigs receiving injectable antibiotic treatment or number of pigs removed from the study between treatments (Data not shown). Even though mycotoxin contaminated corn may have reduced oil content (Bartov, 1985), the chemical analysis of the diets showed no difference in major nutrient values among treatments (Table 2). Therefore, the reduced growth performance is likely not a function of nutrient value differences between the two corn sources, but rather caused by disrupted gastrointestinal function and other negative effects on organs, such as the heart, liver, and kidneys attributed to the increased fumonisin.

The interactive effects of increasing fumonisin on ADG, ADFI, and G:F by week was also assessed. A linear fumonisin concentration × week interaction was observed ($P < 0.001$) for ADG (Figure 1). Increasing fumonisin concentration linearly reduced ADG from d 7 to 14, but not during any other week. A linear fumonisin concentration × week interaction was also observed ($P = 0.008$) for ADFI (Figure 2). Increasing fumonisin concentration linearly reduced ADFI from d 7 to 14 and d 21 to 28, but not from d 0 to 7. A linear fumonisin × week interaction was also observed ($P < 0.001$) for G:F (Figure 3). Increasing fumonisin concentration linearly reduced G:F from d 0 to 7 and d 7 to 14, but not from d 14 to 21 and d 21 to 28. Pigs fed 21.9 mg/kg fumonisin or less had a normal reduction in G:F over the course of the experimental period (Figure 3); however, pigs fed 32.7 or 35.1 mg/kg fumonisin had severe decreases in G:F from d 0 to 7 and d 7 to 14 compared with other treatments. Feed efficiency for these treatments appeared to recover from d 14 to 21 to be similar to pigs fed 21.9 mg/kg fumonisin or less. The

weekly performance suggests that ADG and ADFI was less affected by fumonisin during the first week than during the second week. This indicates that, for this range of fumonisin concentration, it took more than a week to cause significant reductions in growth performance. This suggests that the reduction in growth performance might not be due to the poorer palatability of feed containing high fumonisin, but due to other negative effects caused by fumonisin intoxication. During the last two weeks (d 14 to 28), some recovery of growth performances was observed. This suggests that after two weeks of fumonisin intoxication, pigs fed high fumonisin diets might be able to acclimate to these fumonisin concentrations. However, the mechanism of increasing tolerance is unknown, and the reduction in ADG and subsequent BW loss were not recovered by d 28.

Gbore (2009) fed diets containing cultured fumonisin B1 from 0.2 to 15 mg/kg for 6 weeks and observed similar linear reductions in growth performance (BW, ADG, ADFI, and G:F) and also found delayed sexual maturity with weaned pigs fed high fumonisin levels. A linear reduction in ADG was also observed in 10 kg nursery pigs when fed 0 to 10 mg/kg of purified fumonisin B1 with pigs fed 10 mg/kg fumonisin having increased organ SA:SO ratio (Rotter et al., 1996). Nursery pigs fed diets containing 10 mg/kg of cultured fumonisin B1 for 4 weeks showed a reduction in BW, feed intake, G:F, and an increase in lung and liver lesions (Harvey et al., 2002). In another study, pigs exposed to 25 to 30 mg/kg of cultured fumonisin B1 for 9 d also had a reduction in G:F (Lessard et al., 2009), which matched our finding that G:F was negatively affected in the first week while ADG and ADFI were not. However, Zomborszky et al. used diets for weaned pigs that contained 0 to 40 mg/kg of cultured fumonisin B1 for 4 weeks (Zomborszky et al., 2000) or 0 to 10 mg/kg of cultured fumonisin B1 for 8 weeks (Zomborszky-Kovacs et al., 2002) and observed no evidence of differences in growth performance, even though there were

increasing signs of pathological changes in lungs of some pigs fed more than 10 mg/kg of fumonisin B1.

After fumonisin ingestion and absorption, the toxin will accumulate in tissues, such as lung, liver, kidneys, brain, and fat tissue. Liver and kidneys are the preferable organs for fumonisin B1 accumulation (Fodor et al., 2006). Increased SA and SO can be observed in these tissues with increased fumonisin concentration (Enongene et al., 2002). Sphinganine accumulates in a faster rate than sphingosine, which increases the SA:SO ratio. Generally, serum SA:SO ratios of control pigs without fumonisin intoxication range from undetectable to 1.08 (Hsiao et al., 2007). In our study, all selected pigs had serum SA:SO ratios below 1:1 on d 0. We observed that increasing fumonisin concentration increased (linear, $P < 0.001$) serum SA:SO ratio from 0.47 to 1.40 on d 14 and 0.55 to 1.58 on d 28. By correlating our growth performance results with serum SA:SO ratios, there was a threshold of approximately 20 to 30 mg/kg of dietary fumonisin (B1+B2) that had the greatest reduction in growth performance which corresponded with serum SA:SO ratios over 1:1, the ratio value that is generally classified as fumonisin intoxication. Dilkin et al. (2010) observed plasma SA:SO ratios of 25-kg pigs were increased 12-fold to 1.11:1 at 6 h after a single dose of fumonisin B1 (5 mg/kg of BW, equivalent to 83 mg/kg of feed). This indicated that plasma SA:SO is a quick and sensitive biomarker for fumonisin toxicosis. Schertz et al. (2018) fed 34-kg pigs a single dose of cultured fumonisin (B1+B2) at 2.5 mg/kg BW and observed significantly elevated serum SA:SO ratios at 24 h post dosing and continued to increase at 120 h post dosing (end of the trial) with no evidence of differences in other blood criteria or lung lesions. Zomborszky et al. (2000) fed 0, 10, 20, and 40 mg/kg of cultured fumonisin B1 to 10 kg pigs and observed increasing SA:SO ratios as fumonisin increased on d 3 of the trial, and the degree of difference between treatments increased until the last day (d 8) of the trial. Riley et

al. (1993) also observed that serum SA:SO increased significantly when pigs were fed fumonisin at 23 mg/kg or greater of purified fumonisin (B1+B2) after 5 d, which matched our serum SA:SO results. Even though Riley et al. (1993) observed elevated serum SA:SO ratios, pigs did not develop lesions in liver, lung, and kidney when fed 23 mg/kg of fumonisin (B1+B2). Meanwhile liver lesions were observed when pigs were fed 39 mg/kg or greater of fumonisin (B1+B2) and lung lesions were observed when pigs were fed 175 mg/kg of fumonisin (B1+B2). These results indicate that the higher the fumonisin level, the faster the SA:SO ratio increases. Therefore, serum SA:SO ratio has the potential to be used as a reliable and quick biomarker for fumonisin's negative effect on pig growth performance without euthanizing the animals for autopsy.

In conclusion, our result of feeding pigs with naturally contaminated fumonisin on growth performance and serum SA:SO ratio matches previous studies where pigs fed less than 25 ppm of purified or cultured fumonisin had minimal changes in clinical chemistry (Ensely and Radke, 2019). Diets that contain greater than 32.7 mg/kg of fumonisin (B1+B2) should not be fed to 9- to 28-kg nursery pigs. Furthermore, diets containing greater than 21.9 mg/kg should be evaluated with caution as further research is warranted to determine the fumonisin concentration between 21.9 and 32.7 mg/kg where the negative effects on pig performance and serum SA:SO ratio are observed. Moreover, pigs ingesting fumonisin below 21.9 ppm for greater than 28 d still needs further evaluation to determine the effect of long term fumonisin intoxication.

References

ANKOM Technology. 2004. Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. ANKOM Technology, Fairport, NY.

ANKOM Technology. 2005. Method for Determining Crude Fiber. ANKOM Technology, Fairport, NY.

AOAC Int. 2019. Official methods of analysis AOAC international. 21st ed. Association of Official Analytical Chemists, Arlington, VA.

Bartov, I. 1985. Comparative effects of antifungal compounds on the nutritional value of diets containing moldy corn for broiler chicks. Poult Sci 64(6):1236-1238. doi: 10.3382/ps.0641236

Bouhet, S., E. Hourcade, N. Loiseau, A. Fikry, S. Martinez, M. Roselli, P. Galtier, E. Mengheri, and I. P. Oswald. 2004. The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. Toxicol. Sci. 77(1):165-171. doi: 10.1093/toxsci/kfh006

Bracarense, A. P., J. Lucioli, B. Grenier, G. Drociunas Pacheco, W. D. Moll, G. Schatzmayr, and I. P. Oswald. 2012. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. Br. J. Nutr. 107(12):1776-1786. doi: 10.1017/S0007114511004946

Colvin, B. M., A. J. Cooley, and R. W. Beaver. 1993. Fumonisin toxicosis in swine: clinical and pathologic findings. J. Vet. Diagn. Invest. 5(2):232-241. doi: 10.1177/104063879300500215

Dilkin, P., G. Direito, M. M. Simas, C. A. Mallmann, and B. Correa. 2010. Toxicokinetics and toxicological effects of single oral dose of fumonisin B1 containing Fusarium

verticillioides culture material in weaned piglets. Chem. Biol. Interact. 185(3):157-162. doi: 10.1016/j.cbi.2010.03.025

Enongene, E. N., R. P. Sharma, N. Bhandari, J. D. Miller, F. I. Meredith, K. A. Voss, and R. T. Riley. 2002. Persistence and reversibility of the elevation in free sphingoid bases induced by fumonisin inhibition of ceramide synthase. Toxicol. Sci. 67(2):173-181. doi: 10.1093/toxsci/67.2.173

Ensely, S. M., and S. L. Radke. 2019. Mycotoxins in Grains and Feeds, Diseases of Swine. Wiley-Blackwell, Hoboken, NJ. p. 1055-1071.

European Commission. 2016. Commission Regulation (EU) 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food. Off. J. EU. L (208):58-60.

Fodor, J., K. Meyer, M. Riedlberger, J. Bauer, P. Horn, F. Kovacs, and M. Kovacs. 2006. Distribution and elimination of fumonisin analogues in weaned piglets after oral administration of Fusarium verticillioides fungal culture. Food Addit. Contam. 23(5):492-501. doi: 10.1080/02652030500544964

Gbore, F. A. 2009. Growth performance and puberty attainment in growing pigs fed dietary fumonisin B(1). J. Anim. Physiol. Anim. Nutr. (Berl.) 93(6):761-767. doi: 10.1111/j.1439-0396.2008.00866.x

Harvey, B., T. S. Edrington, L. F. Kubena, G. E. Rottinghaus, J. R. Turk, K. J. Genovese, R. L. Ziprin, and D. J. Nisbet. 2002. Toxicity of fumonisin from Fusarium verticillioides culture material and moniliformin from Fusarium fujikuroi culture material when fed singly and in combination to growing barrows. J. Food Prot. 65(2):373-377. doi: 10.4315/0362-028x-65.2.373

Hendel, E. G., P. N. Gott, S. Curry, U. Hofstetter-Schähs, and G. R. Murugesan. 2020. Trends in Mycotoxin Contamination in United States Corn. Poster session presented at: Midwest ASAS, 2020 Mar 2-4, Omaha, Nebraska.

Hsiao, S. H., M. E. Tumbleson, P. D. Constable, and W. M. Haschek. 2007. Use of formalin-fixed tissues to determine fumonisin B1-induced sphingolipid alterations in swine. J. Vet. Diagn. Invest. 19(4):425-430. doi: 10.1177/104063870701900417

Kovar, J. L. 2003. Method 6.3 Inductively coupled plasma spectroscopy. University of Wisconsin-Extension, Madison, WI.

Lessard, M., G. Boudry, B. Seve, I. P. Oswald, and J. P. Lalles. 2009. Intestinal physiology and peptidase activity in male pigs are modulated by consumption of corn culture extracts containing fumonisins. J. Nutr. 139(7):1303-1307. doi: 10.3945/jn.109.105023

Mateos, I., S. Combes, G. Pascal, L. Cauquil, C. Barilly, A. M. Cossalter, J. Laffitte, S. Botti, P. Pinton, and I. P. Oswald. 2018. Fumonisin-Exposure Impairs Age-Related Ecological Succession of Bacterial Species in Weaned Pig Gut Microbiota. Toxins (Basel) 10(6)doi: 10.3390/toxins10060230

Merrill, A. H., Jr., M. C. Sullards, E. Wang, K. A. Voss, and R. T. Riley. 2001. Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ. Health Perspect. 109 Suppl 2:283-289. doi: 10.1289/ehp.01109s2283

Motelin, G. K., W. M. Haschek, D. K. Ness, W. F. Hall, K. S. Harlin, D. J. Schaeffer, and V. R. Beasley. 1994. Temporal and dose-response features in swine fed corn screenings contaminated with fumonisin mycotoxins. Mycopathologia 126(1):27-40. doi: 10.1007/bf01371170

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing

Riley, R. T., N. H. An, J. L. Showker, H. S. Yoo, W. P. Norred, W. J. Chamberlain, E. Wang, A. H. Merrill, Jr., G. Motelin, V. R. Beasley, and et al. 1993. Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker of exposure to fumonisin-containing feeds in pigs. Toxicol. Appl. Pharmacol. 118(1):105-112. doi: 10.1006/taap.1993.1015

Riley, R. T., E. Wang, and A. H. Merrill, Jr. 1994. Liquid Chromatographic Determination of Sphmgamne and Sphingosine: Use of the Free Sphinganine-to-Sphingosine Ratio as a Biomarker for Consumption of Fumonisins. J. AOAC Int. 77(2):533-540. doi: 10.1093/jaoac/77.2.533

Rotter, B. A., B. K. Thompson, D. B. Prelusky, H. L. Trenholm, B. Stewart, J. D. Miller, and M. E. Savard. 1996. Response of growing swine to dietary exposure to pure fumonisin B1 during an eight-week period: growth and clinical parameters. Nat. Toxins 4(1):42-50. doi: 10.1002/19960401nt6

Schertz, H., J. Kluess, J. Frahm, D. Schatzmayr, I. Dohnal, G. Bichl, H. Schwartz-Zimmermann, G. Breves, and S. Danicke. 2018. Oral and Intravenous Fumonisin Exposure in Pigs-A Single-Dose Treatment Experiment Evaluating Toxicokinetics and Detoxification. Toxins (Basel) 10(4)doi: 10.3390/toxins10040150

Smith, M. C., S. Madec, E. Coton, and N. Hymery. 2016. Natural Co-Occurrence of Mycotoxins in Foods and Feeds and Their in vitro Combined Toxicological Effects. Toxins (Basel) 8(4):94. doi: 10.3390/toxins8040094

U.S. Food & Drug Administration. 2001. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds.

Varga, E., K. Mayer, M. Sulyok, R. Schuhmacher, R. Krska, and F. Berthiller. 2013. Validation of a Stable Isotope Dilution Assay for the Accurate Quantitation of Mycotoxin in Maize Using UHPLC/MS/MS. Agilent Technologies, Inc., Waldbronn, Germany.

Wilson, D. 2012. Fumonisin Toxicosis, Clinical Veterinary Advisor: The Horse. Saunders, Philadelphia, PA. p. 214-215.

Zomborszky-Kovacs, M., F. Vetesi, P. Horn, I. Repa, and F. Kovacs. 2002. Effects of prolonged exposure to low-dose fumonisin B1 in pigs. J. Vet. Med. B Infect. Dis. Vet. Public Health 49(4):197-201. doi: 10.1046/j.1439-0450.2002.00519.x

Zomborszky, M. K., F. Vetesi, I. Repa, F. Kovacs, A. Bata, P. Horn, A. Toth, and R. Romvari. 2000. Experiment to determine limits of tolerance for fumonisin B1 in weaned piglets. J. Vet. Med. B Infect. Dis. Vet. Public Health 47(4):277-286. doi: 10.1046/j.1439-0450.2000.00343.x\

Table 1-1 Diet composition (as-fed basis)[1,2]

	Analyzed fumonisin concentration, mg/kg	
Item	7.2	35.1
Ingredients, %		
Corn, 10 mg/kg fumonisin B1+B2	64.70	--
Corn, 50 mg/kg fumonisin B1+B2[3]	--	64.70
Soybean meal	28.00	28.00
Soybean oil	3.00	3.00
Monocalcium phosphate	0.85	0.85
Calcium carbonate	0.75	0.75
Sodium chloride	0.60	0.60
L-Lysine HCl	0.55	0.55
DL-Methionine	0.21	0.21
L-Threonine	0.23	0.23
L-Tryptophan	0.06	0.06
L-Valine	0.16	0.16
Vitamin premix[4]	0.25	0.25
Trace mineral premix[4]	0.15	0.15
Phytase[5]	0.08	0.08
Total	100	100
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lysine	1.30	1.30
Isoleucine:lysine	53	53
Leucine:lysine	111	111
Methionine:lysine	36	36
Methionine and cysteine:lysine	56	56
Threonine:lysine	63	63
Tryptophan:lysine	20.0	20.0
Valine:lysine	69	69

Histidine:lysine	35	35
Net energy, kcal/kg	2,536	2,536
Crude protein, %	19.8	19.8
Calcium, %	0.61	0.61
STTD P[6], %	0.44	0.44

[1]Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

[2]The 7.2 and 35.1 mg/kg fumonisin treatments were blended to manufacture diets with intermediate fumonisin level (14.7, 21.9, and 32.7 mg/kg of fumonisin).

[3]Approximately 50 mg/kg of fumonisin B1+B2.

[4]Provided per kg complete feed: 4,133 IU vitamin A; 1653 IU vitamin D3; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg d-pantothenic acid; 8 mg riboflavin; 0.30 mg selenium; 16 mg Cu from copper sulfate; 110 mg Fe from iron sulfate; 110 mg Zn from zinc sulfate; 33 mg Mn from Manganese sulfate; 0.3 mg I from Ca iodate.

[5]Ronozyme HiPhos 2700 (DSM Nutritional Products, Basel, Switzerland) provided 2,027 FTU per kg of feed and an expected STTD P release of 0.12%.

[6]STTD P = standardized total tract digestible phosphorus.

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

	Analyzed fumonisin concentration, mg/kg				
Item, %	7.2	14.7	21.9	32.7	35.1
Dry matter	87.54	87.56	87.72	87.92	88.14
Crude protein	19.3	19.3	19.8	19.7	19.8
Ca	0.73	0.65	0.57	0.67	0.63
P	0.51	0.49	0.50	0.51	0.53
Neutral detergent fiber	6.5	5.6	6.6	6.8	6.3
Ether extract	5.1	4.9	4.8	5.0	5.1

[1]A representative sample of each diet was collected from every fifth bag of feed manufactured for each treatment, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE).

[2]Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

Table 1-3 Dietary mycotoxin level (as-fed basis)[1, 2, 3]

	Analyzed fumonisin concentration, mg/kg				
Item, mg/kg	7.2	14.7	21.9	32.7	35.1
Fumonisin B1	5.68	11.71	17.35	25.21	27.46
Fumonisin B2	1.49	2.96	4.51	7.46	7.54

[1]A representative sample of each diet was collected from every fifth bag of feed manufactured for each treatment.

[2]Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

[3]Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, T-2 Toxin, ochratoxin, and sterigmatocystin were below detectable concentration (< 20 ug/kg); HT-2 toxin and vomitoxin were below detectable concentration (< 200 ug/kg); and zearalenone was below detectable concentration (< 100 ug/kg).

Table 1-4 Effect of fumonisin concentration on 9- to 28-kg nursery pig growth performance [1, 2]

Item	Analyzed fumonisin concentration, mg/kg					SEM	Probability, *P* <	
	7.2	14.7	21.9	32.7	35.1		Linear	Quadratic
BW, kg								
d 0	8.9	8.9	8.9	8.9	8.9	<0.21[3]	0.598	0.724
d 28	28.1	27.7	27.8	26.8	26.6	0.42	< 0.001	0.410
d 0 to 28								
ADG, g	677	666	674	640	633	10.4	0.001	0.184
ADFI, g	1,016	993	1,010	974	978	18.6	0.055	0.774
G:F, g/kg	667	672	668	658	648	6.4	0.016	0.114

[1] A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 8.9 kg) were used in a 28-d experiment with 5 pigs per pen and 14 pens per treatment.

[2]ADG = average daily gain. ADFI = average daily feed intake. G:F = feed efficiency.

[3]Heterogenous SEM: 7.2 mg/kg (0.19), 14.7 mg/kg (0.19), 21.9 mg/kg (0.18), 32.7 mg/kg (0.21), and 35.1 mg/kg (0.19).

Table 1-5 Effect of fumonisin concentration on serum sphinganine (SA)-to- sphingosine (SO) ratio[1,2,3]

	Analyzed fumonisin concentration, mg/kg						Probability, <	
Item	7.2	14.7	21.9	32.7	35.1	SEM	Linear	Quadratic
d 14								
SA:SO	0.47	0.84	1.00	1.14	1.40	0.09	< 0.001	0.364
d 28								
SA:SO	0.55	0.77	0.93	1.42	1.58	<0.15[4]	< 0.001	0.143

[1]A total of 350 pigs (241 × 600, DNA, Columbus, NE; initially 8.9 kg) were used in a 28-d experiment with 5 pigs per pen and 14 pens per treatment.

[2]Two pigs per treatment were sampled and analyzed as baseline for all treatments on d 0 (SA/SO = 0.22); 9 pigs per treatment were sampled and analyzed on d 14 and 28.

[3]Serum SA-to-SO ratio was analyzed at University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) by HPLC.

[4]Heterogenous variance: 7.2 mg/kg (0.03), 14.7 mg/kg (0.07), 21.9 mg/kg (0.08), 32.7 mg/kg (0.07), and 35.1 mg/kg (0.15).

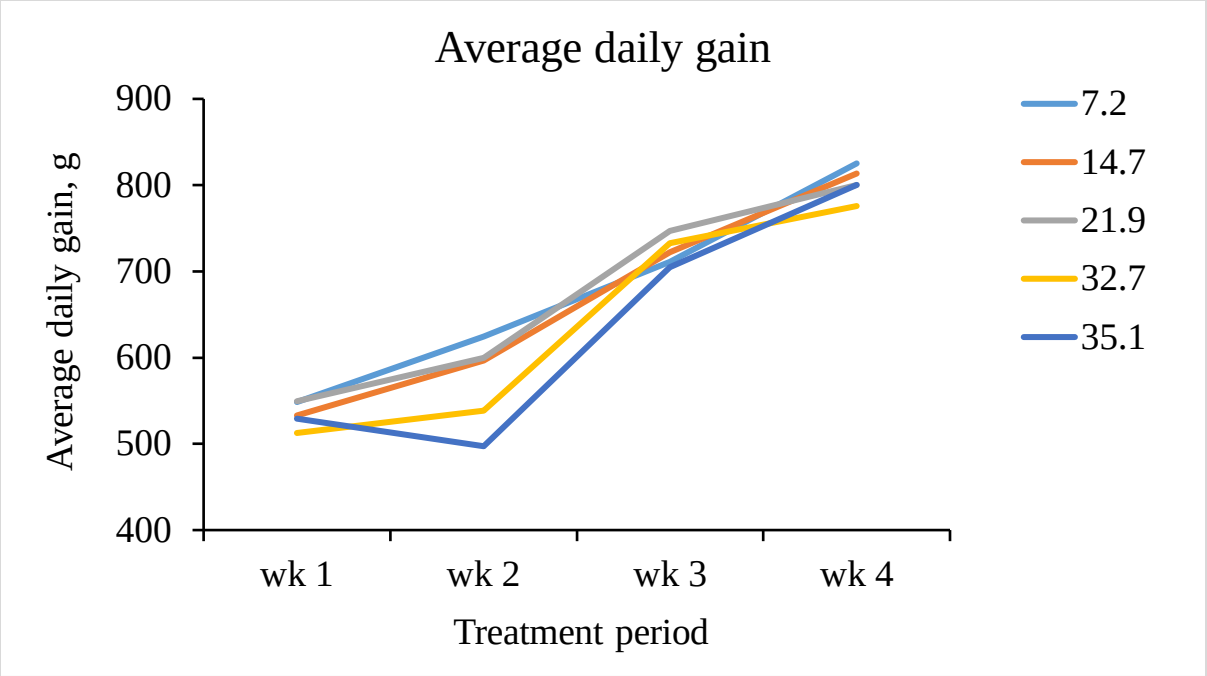

Figure 1-1 Weekly average daily gain of pigs fed increasing fumonisin concentration.

The dietary fumonisin levels were 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg of fumonisin (B1+B2). There was a linear fumonisin concentration × week interactive effect ($P < 0.001$). From d 0 to 7, there was no evidence of linear effect ($P = 0.182$) and quadratic effect ($P = 0.785$). From d 7 to 14, there were a linear effect ($P < 0.001$) and a tendency of quadratic effect ($P = 0.084$). From d 14 to 21, there was no evidence of linear effect ($P = 0.888$), but a tendency of quadratic effect ($P = 0.077$). From d 21 to 28, there was a tendency of linear effect ($P = 0.053$) and no evidence of quadratic effect ($P = 0.615$).

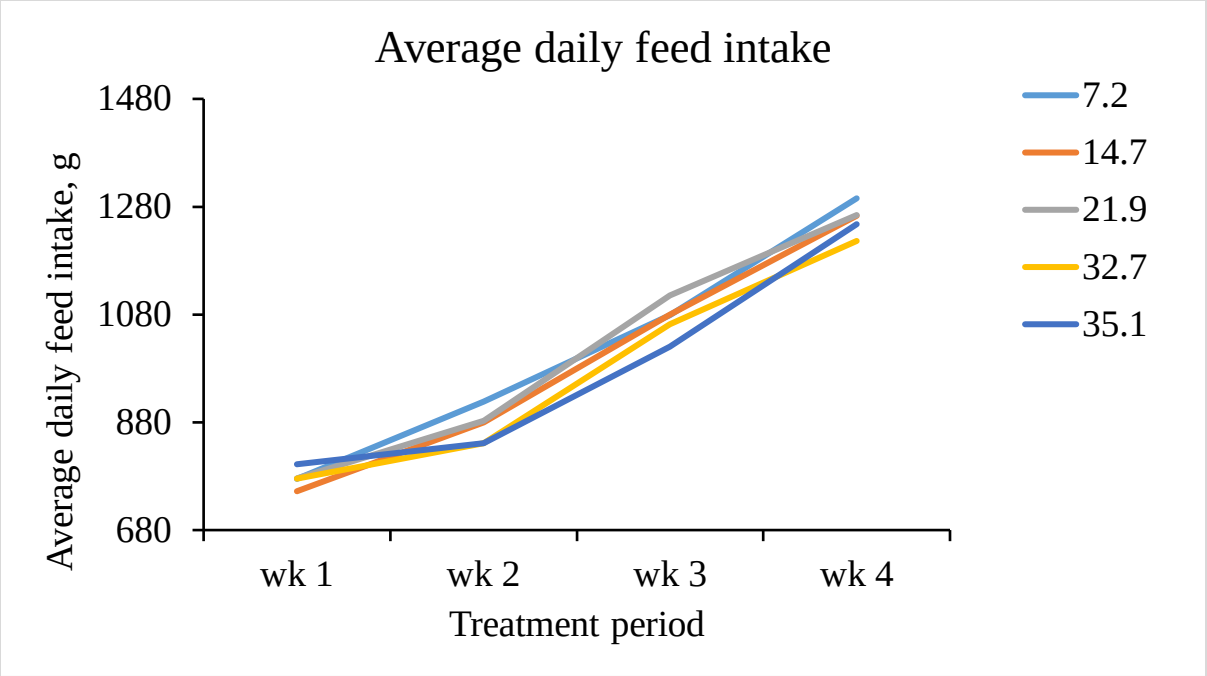

Figure 1-2 Weekly average daily feed intake of pigs fed increasing fumonisin concentration.

The dietary fumonisin levels were 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg of fumonisin (B1+B2). There was a linear fumonisin concentration × week interactive effect (P = 0.008). From d 0 to 7, there was no evidence of linear effect (P = 0.328) or quadratic effect (P = 0.427). From d 7 to 14, there was a linear effect (P = 0.007), but no evidence of quadratic effect (P = 0.892). From d 14 to 21, there were a tendency of linear effect (P = 0.077) and a quadratic effect (P = 0.041). From d 21 to 28, there was a linear effect (P = 0.030), but no evidence of quadratic effect (P = 0.698).

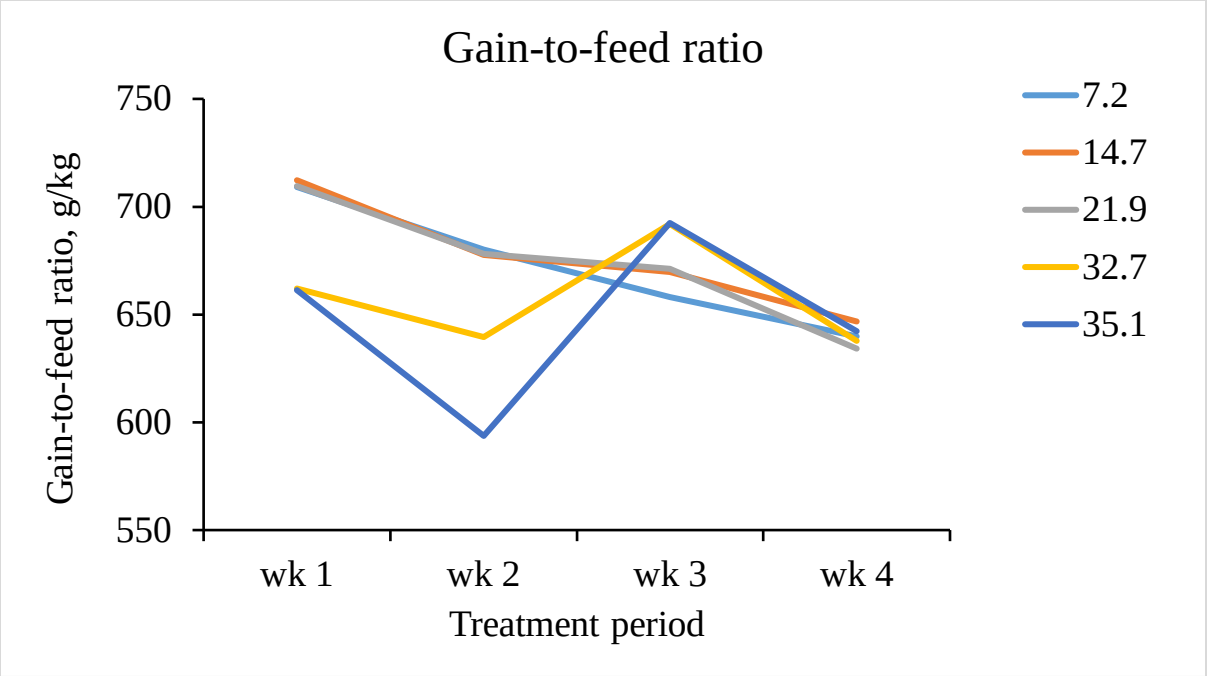

Figure 1-3 Weekly feed efficiency of pigs fed increasing fumonisin concentration.

The dietary fumonisin levels were 7.2, 14.7, 21.9, 32.7, and 35.1 mg/kg of fumonisin (B1+B2). There was a linear fumonisin concentration × week interactive effect ($P < 0.001$). From d 0 to 7, there were a linear effect ($P < 0.001$) and a tendency of quadratic effect ($P = 0.051$). From d 7 to 14, there were a linear effect ($P < 0.001$) and a quadratic effect ($P = 0.004$). From d 14 to 21, there was a linear effect ($P = 0.010$), and no evidence of quadratic effect ($P = 0.826$). From d 21 to 28, there were no linear effect ($P = 0.877$) and quadratic effect ($P = 0.864$).

Chapter 2 - Efficacy of commercial products on nursery pig growth performance fed diets with fumonisin contaminated corn

Abstract

Two experiments were conducted to determine the efficacy of various commercial products on growth performance of nursery pigs fed diets high in fumonisin. In Exp. 1, 350 pigs (241 × 600; DNA, Columbus, NE; initially 9.9 kg) were used with 5 pigs per pen and 14 replicates per treatment. After weaning, pigs were fed common diets for 21 d before the experiment started. The five dietary treatments consisted of a positive control (low fumonisin), a negative control (60 mg/kg of fumonisin B1+B2 in complete diet), and the negative control with 1 of 3 products (0.3% of Kallsil Dry, Kemin Industries Inc., Des Moines, IA; 0.3% of Feed Aid Wide Spectrum, NutriQuest, Mason City, IA; 0.17% of Biofix Select Pro, Biomin America Inc., Overland Park, KS). Diets were fed in mash form for 14 d and followed with a low fumonisin diet for 13 d. For the 14-d treatment period, pigs fed the positive control diet and Biofix Select Pro had greater ($P < 0.05$) average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) compared to those fed the high fumonisin negative control, or high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum. Serum sphinganine to sphingosine ratios (**SA:SO**) were greater ($P < 0.05$) in all pigs fed high fumonisin diets compared to the positive control. In Exp. 2, 300 pigs (241 × 600; DNA; initially 10.4 kg) were used. Procedures were similar to Exp. 1 except there were 12 replicate pens per treatment, high fumonisin diets contained 30 mg/kg fumonisin, and experimental diets were fed for 28 d. Similar to Exp. 1, pigs fed the positive control diet and treatment with Biofix Select Pro had greater ($P < 0.05$) ADG and G:F, and lower ($P < 0.05$) serum SA:SO compared to pigs fed the high fumonisin negative control, or high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum. In summary, pigs

fed diets containing 60 mg/kg of fumonisin for 14 d or 30 mg/kg of fumonisin for 28 d had poorer ADG and G:F and greater serum SA:SO compared to pigs fed a diet with less than 5 mg/kg of fumonisin. Adding Biofix Select Pro to diets appeared to mitigate the negative effects of high fumonisin concentrations, while Kallsil Dry and Feed Aid Wide Spectrum did not.

Keywords: Fumonisin, Growth, Mycotoxin, Nursery pigs, Sphinganine, Sphingosine

Introduction

Fumonisins are a group of toxic secondary metabolites produced mostly by *Fusarium vertcilliodes* and *F. proliferatum*. Fumonisins are highly polar and soluble in water. The fumonisins that cause concern in the animal feed industry are fumonisin B1, B2, and B3, and their concentrations generally appear in an approximate ratio of 3:1:1. In contaminated feed ingredients, fumonisin B1 is most toxic to animals, while fumonisin B2 is secondary, with B3 the least toxic. Fumonisin contamination has been increasing in grains used for animal feed in the US (Hendel et al., 2020). Fumonisin disrupts synthesis of complex sphingolipids, which results in cell damage and the accumulation of sphinganine (**SA**) and sphingosine (**SO**), the precursors of sphingolipids, in tissue. Sphinganine accumulates at a faster rate than SO. Therefore, serum SA:SO ratio has been used as a biomarker to determine the severity of fumonisin intoxication (Zomborszky et al., 2000; Hsiao et al., 2007; Dilkin et al., 2010). Fumonisin toxicosis reduces growth performance and causes damage to intestinal structure, liver, kidneys, and lung (Bracarense et al., 2012; Ensely and Radke, 2019). In a previous study, pigs fed increasing amounts of naturally contaminated fumonisin corn (fumonisin B1+B2 levels of 7.2 to 35.1 mg/kg) resulted in a linear reduction in growth performance and increased serum SA:SO ratio

(Rao et al., 2020). In addition, a SA:SO ratio of over 1:1, which is generally considered as fumonisin intoxication, was observed in pigs fed 32.7 mg/kg fumonisin or greater.

Several commercial products have the potential to mitigate the effects of fumonisin, but little *in vivo* research is available to verify their efficacy. Even though there is no commercial product that is FDA approved for its ability to bind fumonisin, clay-based products are sometimes added to diets to hopefully mitigate the negative effect of fumonisin. Hence, we conducted these experiments to determine the efficacy of commercial products on nursery pig growth performance and serum SA:SO ratio when fed diets with high fumonisin contamination.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments and they were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (1.2 × 1.2 m) provided 0.28 m^2 per pig and was equipped with a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water.

In both experiments, pigs were weaned at approximately 21 d of age and placed in pens of 5 pigs based on initial body weight (BW) and gender. A common phase 1 pelleted diet was fed for 7 d and a common phase 2 mash diet was fed for another 14 d. At d 21 after weaning, which was considered d 0 of the trial, pens of pigs were randomly allotted to treatment in a randomized complete block design with BW as the blocking factor. Diet samples were collected and analyzed for mycotoxins (Table 1 and 2). Diets from Exp. 1 were analyzed for all major mycotoxins without and with a 100× sample dilution, and diets from Exp. 2 were analyzed twice

with 100× sample dilution at the North Dakota State University Veterinary Diagnostic Laboratory (**NDSU**, Fargo, ND). Diets from both experiments were also analyzed with 10× sample dilution for fumonisin at Trilogy laboratory (Washington, MO). Both NDSU and Trilogy laboratories utilized a 10 mg/kg fumonisin standard curve. Diluting samples was required for the high fumonisin levels to be within the standard curve used in each of the laboratories.

The multiple mycotoxin assay conducted at North Dakota State University Veterinary Diagnostic Laboratory was based on an Agilent Technologies (Santa Clara, CA) method for mycotoxin in corn using ultra high pressure liquid chromatography and tandem mass spectrometric detection (**UHPLC/MS/MS**) with modifications (Varga et al., 2013). The detail of this analysis can be found in Rao et al. (2020). The fumonisin analysis conducted at Trilogy laboratories was an internally validated method for 22 mycotoxins, including fumonisin, using LC/MS/MS. For fumonisin analysis, the samples were ground using Retsch GM200 knife mill with serrated blades. The samples were then extracted using official AOAC extractions method (method 995.15; AOAC Inc., 2019) with methanol/water (25g/100 mL, v/v) solution and shook on a laboratory orbital shaker for 1 h. The samples were then filtered with a Whatman No. 1 filter. Extracts were diluted with 1% acetic acid in a 1:4 ratio. Matrix calibration curve was prepared using a known non-detected sample following the above procedure and fortification. The samples were then injected and analyzed on Sciex 6500 Qtrap (AB Sciex LLC, Framingham, MA).

In Exp. 1, a total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 9.9 kg) were used in a 27-d growth trial. There were 5 pigs per pen and 14 replicates per treatment. The five dietary treatments consisted of a positive control (low fumonisin diet, 3 to 4 mg/kg fumonisin B1+B2), a negative control (approximately 50 to 60 mg/kg of fumonisin B1+B2), and 3 other treatments as

negative control with 1 of 3 different commercial products (Kallsil Dry, Kemin Industries Inc., Des Moines, IA; Feed Aid Wide Spectrum, NutriQuest, Mason City, IA; Biofix Select Pro, Biomin America Inc., Overland Park, KS; Table 4). The product inclusion levels were provided by their respective suppliers and were 0.3% for Kallsil Dry and Feed Aid Wide Spectrum and 0.17% for Biofix Select Pro. High fumonisin diets were fed in mash form for 14 d and followed with a low fumonisin (5 mg/kg) common mash diet for 13 d as a post-treatment period. Two diets were formulated using control corn (approximately 5 mg/kg of fumonisin B1+B2) or fumonisin-contaminated corn (approximately 80 mg/kg of fumonisin B1+B2). The diet manufactured with low fumonisin corn was used as the positive control. The diet manufactured with high fumonisin-contaminated corn was manufactured as a single large batch of basal diet. During the bag-off process, each bag was stacked sequentially on 12 numbered pallets to allow fumonisin to be evenly distributed in all treatment diets. Three pallets of bags were randomly selected for each of the 4 fumonisin treatments. Each treatment's basal diet was then mixed again with sand or one of the 3 commercial products to produce the final treatment diet. These experimental diets were fed during the 14-d treatment period. Meanwhile, a low fumonisin diet for the 13-d post treatment period was formulated and manufactured identically to the positive control diet that was used in the 14-d treatment period. Representative diet samples were obtained from every fifth bag of feed manufactured. Pen weights and feed disappearance were measured on d 0, 7, 14, 21, and 27 to determine ADG, ADFI, and G:F.

Based on the results of Exp.1, we were interested in whether the fumonisin products would have better efficacy in diets with lower fumonisin concentrations than those tested in Exp. 1. Therefore, we conducted Exp. 2 with a lower fumonisin concentration (approximately 30 mg/kg of fumonisin B1+B2). In Exp. 2, a total of 300 pigs (241 × 600; DNA; initially 10.4 kg) were

used in a 28-d growth trial with 5 pigs per pen and 12 replicates per treatment. Experimental treatments were the same as Exp. 1, with the exception that the high fumonisin diets only contained approximately 30 mg/kg fumonisin B1+B2 (Table 2). For Exp. 2, a low fumonisin common diet was manufactured identical to the positive control diet in Exp. 1 to be used as the positive control (Table 4). The remaining high fumonisin diets (approximately 50 to 60 mg/kg of fumonisin B1+B2) that were manufactured in Exp. 1 were blended with the positive control diet to produce treatment diets with approximately 30 mg/kg of fumonisin (B1+B2). During blending, each treatment diet was mixed with sand or 1 of the 3 commercial products to produce the same product inclusion level as Exp. 1. Representative diet samples were obtained from 24 bags per treatment. Pen weights and feed disappearance were measured on d 0, 7, 14, 21, and 28 to determine ADG, ADFI, and G:F. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. All diets met or exceeded the NRC (2012) nutrient requirement estimates.

Serum SA and SO analysis

Serum samples were collected on d 0, 14, and the last day of trial for Exp. 1 and 2 to determine serum sphinganine (SA) and sphingosine (SO) ratio. For serum samples, two pigs per treatment were bled and serum analyzed as baseline for all treatments on d 0. Nine pigs per treatment were bled and serum analyzed on d 14 and the last day of both trials. For each selected pen, the median weight pig was selected and recorded on d 0. The same pig was used in all serum collection time points. Serum SA and SO was analyzed at the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) by HPLC as previously described by Rao et al. (2020). Briefly, whole blood samples were allowed to clot for 30 min, centrifuged at

1,500 × g for 15 min. and the resulting serum supernatants transferred to polypropylene tubes and stored at -80°C. Serum samples were sent to the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) and analyzed for sphinganine/sphingosine by HPLC with fluorescence detection utilizing a modification of the method of Hsiao et al (2007) and Riley et al. (1994).

Statistical analysis

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. Models were used to account for heterogeneous variance when appropriate via Bayesian information criterion. Pairwise comparisons were conducted on treatment means using a Tukey adjustment to prevent inflation of Type I error due to multiple comparisons. Data were analyzed using the R program (R Core Team, 2019). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Fumonisin analysis

For Exp. 1, complete diet samples were analyzed twice at NDSU with or without 100× dilution, and once at Trilogy laboratories with 10× dilution. For results from NDSU, the average fumonisin level (B1+B2) was 61.6 mg/kg when analyzed without dilution, and 56.2 mg/kg when analyzed after dilution. The ratios of fumonisin B1 to B2 before and after dilution were both approximately 4:1 for each individual treatment sample. For the samples analyzed at Trilogy laboratories, the average fumonisin levels were 43.1 mg/kg (B1+B2) and 47.7 mg/kg

(B1+B2+B3). The ratio of B1 to B2 was approximately 5.5:1 and consistent for each treatment sample.

For Exp. 2, the complete diet samples were analyzed twice at NDSU with 100× dilution, and once at Trilogy laboratories with 10× dilution. The average fumonisin levels (B1+B2) were 29.3 and 29.5 mg/kg for NDSU, and 23.0 mg/kg for Trilogy laboratories for the high fumonisin diets. The B1 to B2 ratios ranged from 3.2 to 4.1:1 for the first NDSU analysis, 3.4 to 3.8:1 for the second NDSU analysis, and 3.9 to 5.4:1 for the analysis at Trilogy lab.

Experiment 1

From d 0 to 14 (treatment period), pigs fed the low fumonisin positive control diet or high fumonisin diet with Biofix Select Pro had greater ($P < 0.05$) ADG, ADFI, d 14 BW, and G:F compared to those fed the negative control and high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum (Table 5). There was no evidence of differences ($P > 0.05$) between the positive control and high fumonisin diet with Biofix Select Pro for any growth performance criteria. There was no evidence of differences ($P > 0.05$) between the high fumonisin negative control and high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum for any growth performance criteria. However, serum SA:SO of all high fumonisin diets were higher ($P < 0.05$) than the positive control with no influence from commercial product inclusion.

During the 13-d post-treatment period, pigs previously fed the high fumonisin negative control diet, or high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum had increased ($P < 0.05$) G:F compared with pigs previously fed the low fumonisin diet or high fumonisin diet with Biofix Select Pro. On d 27, the serum SA:SO ratios of pigs fed high fumonisin diets fell

below 1.0 and there was no evidence of difference between treatments. Final BW on d 27 BW were approximately 3 kg lighter ($P < 0.05$) when fed the high fumonisin negative control diet or high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum compared to pigs fed the positive control diet and high fumonisin diet with Biofix Select Pro.

From d 0 to 27 (overall period), pigs fed the low fumonisin positive control diet or high fumonisin diet with Biofix Select Pro had greater ($P < 0.05$) ADG, ADFI, and d 27 BW compared to those fed the negative control and high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum. Pigs fed Biofix Select Pro had improved ($P < 0.05$) G:F compared to those fed the high fumonisin negative control diet or high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum. There was no evidence of differences ($P > 0.05$) between the positive control and high fumonisin diet with Biofix Select Pro for any growth performance criteria. There was no evidence of differences ($P > 0.05$) between the high fumonisin negative control and high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum on any growth performance criteria.

Experiment 2

Similar to Exp. 1, from d 0 to 28, pigs fed the low fumonisin positive control diet or high fumonisin diet with Biofix Select Pro had greater ($P < 0.05$) ADG, d 28 BW, and G:F compared to those fed the negative control and high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum (Table 6). Average daily feed intake was highest ($P < 0.05$) for those fed the diet with Biofix Select Pro compared to the negative control or negative control with Feed Aid Wide Spectrum, with others intermediate. Serum SA:SO measured on d 14 and 28 was lower ($P < 0.05$) for the positive control and pigs fed the diet containing Biofix Select Pro compared to

those fed the negative control and high fumonisin diets with Kallsil Dry or Feed Aid Wide Spectrum. There was no evidence of differences ($P > 0.05$) between pigs fed the positive control and those fed the high fumonisin diet with Biofix Select Pro for any growth performance and serum criteria. There was no evidence of differences ($P > 0.05$) between pigs fed the high fumonisin negative control and those fed high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum in any growth performance and serum criteria.

Discussion

Mycotoxins generally have uneven distribution and low concentration in feed ingredients (Zhang et al., 2018), therefore there can be variation during feed manufacturing on dietary fumonisin analysis. Our treatment diets for both experiments were analyzed multiple times for fumonisin levels at North Dakota State University (B1, B2) and Trilogy laboratories (B1, B2, and B3). Although variation in analysis exists between laboratories, the results clearly show the experimental design was successful in creating the desired low and high fumonisin diets with similar fumonisin levels within the high fumonisin diets containing the different products tested.

Increasing prevalence and level of fumonisin contamination in corn has been an emerging issue for swine feed production (Hendel et al., 2020). Although fumonisin has a low absorption rate, can be partially metabolized by porcine microbiota (Fodor et al., 2008; Schertz et al., 2018), and most of the toxin is excreted in feces (Dilkin et al., 2010), high fumonisin concentrations can still cause hepatic and intestinal damage (Motelin et al., 1994; Bouhet and Oswald, 2007; Yuan et al., 2019), and reduce the growth performance of animals (Harvey et al., 2002; Gbore, 2009; Lessard et al., 2009; Rao et al., 2020). The U.S. Food and Drug Administration (**FDA**) suggests a

guidance of maximum fumonisin (B1+B2+B3) level of 20 mg/kg in corn used in animal feed. Furthermore, at this level of contamination, the corn should not exceed 50% in swine diets (U.S. Food & Drug Administration, 2001). The European Commission also established a fumonisin recommendation for corn used in animal feed to be below 60 mg/kg of fumonisin (B1+B2) and no more than 5 mg/kg in complete swine diets (European Commission, 2016). In 2009, EU approved a new functional feed additive group for mycotoxin detoxifying agents (European Commission, 2009); however, these feed additives, which aim to reduce mycotoxin's negative effects, are not approved to be used as such in the U.S. These mycotoxin-detoxifying agents have two main modes of action. They act by either binding the mycotoxins (adsorbing agents) or transforming the mycotoxins into less toxic forms (bio-transforming agents). For the U.S. market, clay based adsorbing agents are the main products used in the animal feed industry and were used in our two experiments.

Adsorbing agents have high molecular weight with specific physical properties (total charge, polarity, and porosity) that bind mycotoxins with hydrophobic binding, hydrogen bonds, electrostatic attraction or repulsion, or coordination bond to form mycotoxin-adsorbing agent complexes. Mycotoxin-adsorbing agent complexes needs to be stable throughout the digestive tract, which has varying pH conditions, in order to reduce the absorption of mycotoxins and eventually be excreted into feces. Different mycotoxins have different physiochemical characteristics (polarity, solubility, and structure). Therefore, one adsorbing agent does not necessarily work with multiple mycotoxins, but usually on one specific kind of mycotoxin. Also, it should be noted that these adsorbing agents, especially activated carbon, may bind to micronutrients, such as minerals and vitamins, therefore reducing the bioavailability of these nutrients (Kolosova and Stroka, 2011). There are several kinds of inorganic and organic

adsorbing agents, such as hydrated sodium calcium aluminosilicates (**HSCAS**), bentonites, zeolites, yeast cell wall, lactic acid bacteria, micronized fiber, activated carbon, organoaluminosilicates, modified clay, and polymers (Vila-Donat et al., 2018). The 3 commercial products used in these two trials were all categorized as nonmodified clay-based mycotoxin adsorbing agents, which adsorb fumonisin with their physical property and structure (Vila-Donat et al., 2018). Kallsil Dry (Kemin Industries Inc., Des Moines, IA) is labeled as HSCAS and mineral oil. Feed Aid Wide Spectrum (NutriQuest, Mason City, IA) is labeled as bentonite, sodium metabisulfite, and mineral oil. Biofix Select Pro (Biomin America Inc., Overland Park, KS) is a combination of active dry yeast, yeast culture, bentonite, diatomaceous earth, dry kelp, and a natural flavoring compound. Hydrated sodium calcium aluminosilicates can effectively bind with aflatoxins (Kabak et al., 2006; Harper et al., 2010), but failed to be effective on fumonisin or trichothecenes (Vila-Donat et al., 2018). Bentonites are effective for binding aflatoxins and zearalenone (Miazzo et al., 2005; Wang et al., 2012), but in an earlier study was not effective against fumonisin (Avantaggiato et al., 2005). Sodium metabisulfite can restore the performance of pigs from DON contaminated feed (Frobose et al., 2017; Shawk et al., 2018), but no published research has been conducted to test its efficacy against fumonisin. Yeast cell wall has a large capability to adsorb a wide spectrum of mycotoxins, such as aflatoxin, zearalenone, and ochratoxin A (Fruhauf et al., 2012; Pfohl-Leszkowicz et al., 2015), but there is limited information on fumonisin (Pfliegler et al., 2015). Diatomaceous earth restores growth performance and reduces liver damage of broiler chicks fed aflatoxin (Modirsanei et al., 2008; Lakkawar et al., 2017), but no published results can confirm its effect on pigs fed fumonisin contaminated grain. Most studies with fumonisin detoxifying agents have been conducted *in vitro*, and little in vivo data is available in any animal models, let alone pigs.

The negative effect of fumonisin toxicosis is mainly caused by the competitive inhibition between fumonisin and sphinganine. They have similar molecule structures which compete for ceramide synthase, an important enzyme for *de novo* sphingolipid biosynthesis. Therefore, fumonisin disrupts the metabolism of complex sphingolipids that are used in lipid-rich components, such as cell membrane. Without ceramide synthase, the conversion of sphinganine and sphingosine to sphingolipids is disrupted, so SA and SO concentration increases in animal tissue, especially in liver and kidneys. Sphinganine increases at a faster rate than sphingosine, therefore SA:SO ratio has been used as a biomarker for fumonisin toxicosis. Increased SA and SO concentrations are cytotoxic and reduced synthesis of sphingolipids cause cell apoptosis (Merrill et al., 2001). Generally, serum SA:SO ratios above 1:1 are considered fumonisin toxicosis (Hsiao et al., 2007). In Exp. 1, even though there was no evidence of difference in growth performance between pigs fed the positive control treatment and those fed high fumonisin diets containing Biofix Select Pro, the high fumonisin still elicited negative effects on d 14 serum SA:SO. This could indicate that SA:SO is a more sensitive indicator of fumonisin contamination than growth performance.

In a previous study, we observed a linear reduction in growth performance when pigs were fed diets contaminated with 7.2 to 35.1 mg/kg of fumonisin (B1+B2; Rao et. al., 2020). Furthermore, serum SA:SO ratios were increased over 1:1 in pigs fed dietary fumonisin level above 32.7 mg/kg. The corn used in the two studies herein came from the same source as the corn used in our previous study, which contained high naturally contaminated fumonisin without other mycotoxins (Table 1 and 2). This allowed us to determine the effect of fumonisin without the interactive effect of multiple mycotoxins. According to the limited literature on mycotoxin-detoxifying agents on fumonisin, the components of these products do not have well established

evidence on binding fumonisin. However, our result suggested that adding Biofix Select Pro in high fumonisin diets reversed the negative effect of fumonisin and had no evidence of difference compared to pigs fed the low fumonisin positive control. The improved performance may due the inclusion of yeast product that may have different properties compared to those in previous research, but the mechanism is unclear. A fumonisin esterase enzyme, which is produced by yeast, has been developed that biotransforms fumonisin into a non-toxic metabolite (European Commission, 2014; Schertz et al., 2018). This enzyme can only be added to diets in purified form in countries where it has been approved. Further research is needed to determine the mode of action of Biofix Select Pro on mitigating the negative effect of fumonisin.

The magnitude of growth reduction caused by fumonisin was different between Exp. 1 and 2. Pigs fed 60 mg/kg of fumonisin for 2 weeks in Exp. 1 had approximately 48% reduction in ADG, while pigs fed 30 mg/kg of fumonisin for 4 weeks in Exp. 2 had approximately 15% reduction in ADG. However, in a previous study, pigs fed similar concentration of fumonisin as Exp. 2 (30 to 35 mg/kg of fumonisin) for 4 weeks had approximately 6% reduction in ADG (Rao et al., 2020). We observed icteric serum (Figure 1), a sign of hepatic damage, in pigs fed the high fumonisin negative control diet and high fumonisin diet with Kallsil Dry or Feed Aid Wide Spectrum in Exp. 1 (60 mg/kg of fumonisin). Colvin et al. (1993) also observed icterus in pigs fed 200 mg/kg of fumonisin B1 for 4 d and pigs intubated with 4 to 16 mg/kg BW of fumonisin B1. Pigs fed 75 to 100 mg/kg of fumonisin for 1 to 3 weeks developed icterus without development of pulmonary edema (Osweiler et al., 1993). Serum of pigs fed the low fumonisin positive control were normal, while serum of pigs fed the high fumonisin diet with Biofix Select Pro were slightly yellow.

Recovery of fumonisin intoxication

In Exp. 1, after 14 d of the high fumonisin treatment diets, all pigs were fed a low fumonisin diet in order to observe the recovery from fumonisin contamination. We observed a recovery of growth performance on pigs previously affected by fumonisin, but they did not compensate to the same performance as pigs fed the low fumonisin diet on d 27. The recovery was not just observed on growth performance, but also in SA:SO ratios. Icteric serum, which was observed in d 14 serum samples, was not observed in d 27 serum samples. The d 27 serum SA:SO ratios were reduced to below 1:1 ratio indicating that there was minimal fumonisin intoxication. These results suggest that pigs experienced a recovery from fumonisin toxicosis after a short-term (14 d) exposure to 50 to 60 mg/kg of fumonisin; however, the pigs did not recover to similar BW as the positive control in the 13-d post-treatment period. Whether pigs previously affected by high fumonisin can recover to the same performance as non-affected pigs needs further research. These results match the literature on fumonisin metabolism. The biodistribution and pharmacokinetics of fumonisin in pigs indicates a rapid absorption and rapid elimination through feces, but a low residue would remain in tissue, especially in liver and kidneys because of their high affinity toward fumonisin. This long half-life is primarily attributed to the enterohepatic recirculation of fumonisin (Prelusky et al., 1994; 1996). Therefore, withdrawing fumonisin contaminated feed from pigs previously fed 14 days of high fumonisin (60 mg/kg) and offering a diet low in fumonisin would reduce the negative effect of fumonisin within a short period of time (13 days), but pigs may still have a low tissue residual that does not have evidence to affect pig's serum SA:SO ratio. Also, the length of recovery period for pigs fed high fumonisin for more than 14 days needs further research.

In conclusion, feeding high fumonisin contaminated diets reduced growth performance and increased the SA:SO ratio. Adding Biofix Select Pro to diets appeared to mitigate the negative effects of high fumonisin concentrations, while Kallsil Dry and Feed Aid Wide Spectrum did not. The SA:SO ratio appears to be a sensitive indicator of fumonisin contamination.

References

AOAC Int. 2019. Official methods of analysis AOAC international. 21st ed. Association of Official Analytical Chemists, Arlington, VA.

Avantaggiato, G., M. Solfrizzo, and A. Visconti. 2005. Recent advances on the use of adsorbent materials for detoxification of Fusarium mycotoxins. Food Addit. Contam. 22(4):379-388. doi: 10.1080/02652030500058312

Bouhet, S., and I. P. Oswald. 2007. The intestine as a possible target for fumonisin toxicity. Mol. Nutr. Food Res. 51(8):925-931. doi: 10.1002/mnfr.200600266

Bracarense, A. P., J. Lucioli, B. Grenier, G. Drociunas Pacheco, W. D. Moll, G. Schatzmayr, and I. P. Oswald. 2012. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. Br. J. Nutr. 107(12):1776-1786. doi: 10.1017/S0007114511004946

Colvin, B. M., A. J. Cooley, and R. W. Beaver. 1993. Fumonisin toxicosis in swine: clinical and pathologic findings. J. Vet. Diagn. Invest. 5(2):232-241. doi: 10.1177/104063879300500215

Dilkin, P., G. Direito, M. M. Simas, C. A. Mallmann, and B. Correa. 2010. Toxicokinetics and toxicological effects of single oral dose of fumonisin B1 containing Fusarium verticillioides culture material in weaned piglets. Chem. Biol. Interact. 185(3):157-162. doi: 10.1016/j.cbi.2010.03.025

Ensely, S. M., and S. L. Radke. 2019. Mycotoxins in Grains and Feeds, Diseases of Swine. Wiley-Blackwell, Hoboken, NJ. p. 1055-1071.

European Commission. 2009. Commission regulation (EC) No 386/2009 of 12 May 2009 amending Regulation (EC) No 1831/2003 of the European Parliament and of the Council

as regards the establishment of a new functional group of feed additives. Off. J. EU. L (118):66.

European Commission. 2014. COMMISSION IMPLEMENTING REGULATION (EU) No 1115/2014 of 21 October 2014 concerning the authorisation of a preparation of fumonisin esterase produced by Komagataella pastoris (DSM 26643) as a feed additive for pigs Off. J. EU. L. (302):51-53.

European Commission. 2016. Commission Regulation (EU) 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food. Off. J. EU. L (208):58-60.

Fodor, J., K. Balogh, M. Weber, M. Miklos, L. Kametler, R. Posa, R. Mamet, J. Bauer, P. Horn, F. Kovacs, and M. Kovacs. 2008. Absorption, distribution and elimination of fumonisin B(1) metabolites in weaned piglets. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25(1):88-96. doi: 10.1080/02652030701546180

Frobose, H. L., E. W. Stephenson, M. D. Tokach, J. M. DeRouchey, J. C. Woodworth, S. S. Dritz, and R. D. Goodband. 2017. Effects of potential detoxifying agents on growth performance and deoxynivalenol (DON) urinary balance characteristics of nursery pigs fed DON-contaminated wheat. J. Anim. Sci. 95(1):327-337. doi: 10.2527/jas.2016.0664

Fruhauf, S., H. Schwartz, F. Ottner, R. Krska, and E. Vekiru. 2012. Yeast cell based feed additives: studies on aflatoxin B1 and zearalenone. Food Addit. Contam. 29(2):217-231. doi: 10.1080/19440049.2011.630679

Gbore, F. A. 2009. Growth performance and puberty attainment in growing pigs fed dietary fumonisin B(1). J. Anim. Physiol. Anim. Nutr. (Berl.) 93(6):761-767. doi: 10.1111/j.1439-0396.2008.00866.x

Harper, A. F., M. J. Estienne, J. B. Meldrum, R. J. Harrell, and D. E. Diaz. 2010. Assessment of a hydrated sodium calcium aluminosilicate agent and antioxidant blend for mitigation of aflatoxininduced physiological alterations in pigs. J. Swine Health Prod. 18(6):282-289. (Article)

Harvey, B., T. S. Edrington, L. F. Kubena, G. E. Rottinghaus, J. R. Turk, K. J. Genovese, R. L. Ziprin, and D. J. Nisbet. 2002. Toxicity of fumonisin from Fusarium verticillioides culture material and moniliformin from Fusarium fujikuroi culture material when fed singly and in combination to growing barrows. J. Food Prot. 65(2):373-377. doi: 10.4315/0362-028x-65.2.373

Hendel, E. G., P. N. Gott, S. Curry, U. Hofstetter-Schähs, and G. R. Murugesan. 2020. Trends in Mycotoxin Contamination in United States Corn. Poster session presented at: Midwest ASAS, 2020 Mar 2-4, Omaha, Nebraska.

Hsiao, S. H., M. E. Tumbleson, P. D. Constable, and W. M. Haschek. 2007. Use of formalin-fixed tissues to determine fumonisin B1-induced sphingolipid alterations in swine. J. Vet. Diagn. Invest. 19(4):425-430. doi: 10.1177/104063870701900417

Kabak, B., A. D. Dobson, and I. Var. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit. Rev. Food Sci. Nutr. 46(8):593-619. doi: 10.1080/10408390500436185

Kolosova, A., and J. Stroka. 2011. Substances for reduction of the contamination of feed by mycotoxins: A review. World Mycotoxin J. 4(3):225-256. (Review) doi: 10.3920/WMJ2011.1288

Lakkawar, A., H. Narayanaswamy, and M. Satyanarayana. 2017. Study on Efficacy of Diatomaceous Earth to Ameliorate Aflatoxin Induced Patho-Morphological Changes in

Liver and Intestines of Broiler Chicken. Int. J. Livest. Res. 66:1. doi: 10.5455/ijlr.20170520051257

Lessard, M., G. Boudry, B. Seve, I. P. Oswald, and J. P. Lalles. 2009. Intestinal physiology and peptidase activity in male pigs are modulated by consumption of corn culture extracts containing fumonisins. J. Nutr. 139(7):1303-1307. doi: 10.3945/jn.109.105023

Merrill, A. H., Jr., M. C. Sullards, E. Wang, K. A. Voss, and R. T. Riley. 2001. Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ. Health Perspect. 109 Suppl 2:283-289. doi: 10.1289/ehp.01109s2283

Miazzo, R., M. F. Peralta, C. Magnoli, M. Salvano, S. Ferrero, S. M. Chiacchiera, E. C. Q. Carvalho, C. A. R. Rosa, and A. Dalcero. 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poult. Sci. 84(1):1-8. doi: https://doi.org/10.1093/ps/84.1.1

Modirsanei, M., B. Mansoori, A. R. Khosravi, M. M. Kiaei, P. Khazraeinia, M. Farkhoy, and Z. Masoumi. 2008. Effect of diatomaceous earth on the performance and blood variables of broiler chicks during experimental aflatoxicosis. J. Sci. Food Agric. 88(4):626-632. doi: 10.1002/jsfa.3127

Motelin, G. K., W. M. Haschek, D. K. Ness, W. F. Hall, K. S. Harlin, D. J. Schaeffer, and V. R. Beasley. 1994. Temporal and dose-response features in swine fed corn screenings contaminated with fumonisin mycotoxins. Mycopathologia 126(1):27-40. doi: 10.1007/bf01371170

Osweiler, G. D., M. E. Kehrli, J. R. Stabel, J. R. Thurston, P. F. Ross, and T. M. Wilson. 1993. Effects of fumonisin-contaminated corn screenings on growth and health of feeder calves. J. Anim. Sci. 71(2):459-466. doi: 10.2527/1993.712459x

Pfliegler, W. P., T. Pusztahelyi, and I. Pócsi. 2015. Mycotoxins – prevention and decontamination by yeasts. J. Basic Microbiol. 55(7):805-818. doi: 10.1002/jobm.201400833

Pfohl-Leszkowicz, A., K. Hadjeba-Medjdoub, N. Ballet, J. Schrickx, and J. Fink-Gremmels. 2015. Assessment and characterisation of yeast-based products intended to mitigate ochratoxin exposure using in vitro and in vivo models. Food Addit. Contam. 32(4):604-616. doi: 10.1080/19440049.2014.970590

Prelusky, D. B., H. L. Trenholm, B. A. Rotter, J. D. Miller, M. E. Savard, J. M. Yeung, and P. M. Scott. 1996. Biological fate of fumonisin B1 in food-producing animals. Adv. Exp. Med. Biol. 392:265-278. doi: 10.1007/978-1-4899-1379-1_23

Prelusky, D. B., H. L. Trenholm, and M. E. Savard. 1994. Pharmacokinetic fate of 14C-labelled fumonisin B1 in swine. Nat. Toxins 2(2):73-80. doi: 10.1002/nt.2620020205

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing

Rao, Z. X., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, H. Calderon Cartagena, and S. S. Dritz. 2020. Effects of fumonisin-contaminated corn on growth performance of 9- to 28-kg nursery pigs: in press. Master thesis, Kansas State University, Manhattan, KS.

Riley, R. T., E. Wang, and A. H. Merrill, Jr. 1994. Liquid Chromatographic Determination of Sphmgamne and Sphingosine: Use of the Free Sphinganine-to-Sphingosine Ratio as a Biomarker for Consumption of Fumonisins. J. AOAC Int. 77(2):533-540. doi: 10.1093/jaoac/77.2.533

Schertz, H., J. Kluess, J. Frahm, D. Schatzmayr, I. Dohnal, G. Bichl, H. Schwartz-Zimmermann, G. Breves, and S. Danicke. 2018. Oral and Intravenous Fumonisin Exposure in Pigs-A Single-Dose Treatment Experiment Evaluating Toxicokinetics and Detoxification. Toxins (Basel) 10(4)doi: 10.3390/toxins10040150

Shawk, D. J., S. S. Dritz, R. D. Goodband, M. D. Tokach, J. C. Woodworth, and J. M. DeRouchey. 2018. Effects of sodium metabisulfite additives on nursery pig growth. Transl. Anim. Sci. 3(1):103-112. doi: 10.1093/tas/txy098

U.S. Food & Drug Administration. 2001. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds.

Varga, E., K. Mayer, M. Sulyok, R. Schuhmacher, R. Krska, and F. Berthiller. 2013. Validation of a Stable Isotope Dilution Assay for the Accurate Quantitation of Mycotoxin in Maize Using UHPLC/MS/MS. Agilent Technologies, Inc., Waldbronn, Germany.

Vila-Donat, P., S. Marín, V. Sanchis, and A. J. Ramos. 2018. A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. Food Chem. Toxicol. 114:246-259. doi: https://doi.org/10.1016/j.fct.2018.02.044

Wang, J. P., F. Chi, and I. H. Kim. 2012. Effects of montmorillonite clay on growth performance, nutrient digestibility, vulva size, faecal microflora, and oxidative stress in weaning gilts challenged with zearalenone. Anim. Feed Sci. Technol. 178(3):158-166. doi: https://doi.org/10.1016/j.anifeedsci.2012.09.004

Yuan, Q., Y. Jiang, Y. Fan, Y. Ma, H. Lei, and J. Su. 2019. Fumonisin B1 Induces Oxidative Stress and Breaks Barrier Functions in Pig Iliac Endothelium Cells. Toxins (Basel) 11(7)doi: 10.3390/toxins11070387

Zhang, L., X. W. Dou, C. Zhang, A. F. Logrieco, and M. H. A. C. Yang. 2018. A Review of Current Methods for Analysis of Mycotoxins in Herbal Medicines. Toxins (Basel) 10(2):65. doi: 10.3390/toxins10020065

Zomborszky, M. K., F. Vetesi, I. Repa, F. Kovacs, A. Bata, P. Horn, A. Toth, and R. Romvari. 2000. Experiment to determine limits of tolerance for fumonisin B1 in weaned piglets. J. Vet. Med. B Infect. Dis. Vet. Public Health 47(4):277-286. doi: 10.1046/j.1439-0450.2000.00343.x

Table 2-1 Dietary mycotoxin levels, Exp. 1 (as-fed basis, mg/kg)[1, 2]

Item	Positive control	Negative control	Negative control with Kallsil Dry[3]	Negative control with Feed Aid Wide Spectrum	Negative control with Biofix Select Pro
Fumonisin B1					
NDSU (undiluted)	2.33	49.36	48.43	49.39	50.48
NDSU (diluted 100×)	3.07	46.76	42.95	47.89	40.58
Trilogy (diluted 10×)	1.80	35.10	36.80	37.00	37.00
Fumonisin B2					
NDSU (undiluted)	0.57	11.95	12.13	12.15	12.70
NDSU (diluted 100×)	0.97	11.44	11.17	12.92	11.22
Trilogy (diluted 10×)	0.10	5.90	6.70	7.10	6.90
Fumonisin B3					
Trilogy (diluted 10×)	0.20	4.20	4.50	4.80	4.60

[1]A representative sample of each diet was collected from every fifth bag of feed manufactured for each treatment.

[2]All mycotoxins were analyzed at NDSU (Fargo, ND) by LC/MS/MS. The standard curve for fumonisin was up to 10 ppm. Fumonisin B1, B2 and B3 were analyzed at Trilogy lab (Washington, MO) by LC/MS/MS. The standard curve for fumonisin was up to 10 ppm. Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, T-2 Toxin, ochratoxin, and sterigmatocystin were below detectable concentration (< 20 ug/kg); HT-2 toxin and vomitoxin were below detectable concentration (< 200 ug/kg); and zearalenone was below detectable concentration (< 100 ug/kg).

[3] Kallsil Dry (Kemin Industries Inc., Des Moines, IA); Feed Aid Wide Spectrum (NutriQuest, Mason City, IA); and Biofix Select Pro (Biomin America Inc., Overland Park, KS).

Table 2-2 Dietary mycotoxin levels, Exp. 2 (as-fed basis, mg/kg)[1, 2]

Item	Positive control	Negative control	Negative control with Feed Aid Kallsil Dry[3]	Negative control with Feed Aid Wide Spectrum	Negative control with Feed Aid Biofix Select Pro
Fumonisin B1					
NDSU 1st	4.10	24.96	25.10	21.41	20.59
NDSU 2nd	3.24	23.83	23.73	21.26	23.45
Trilogy	2.70	18.90	19.10	17.30	19.20
Fumonisin B2					
NDSU 1st	1.07	6.06	7.31	5.17	6.52
NDSU 2nd	0.96	6.65	7.06	5.63	6.53
Trilogy	0.50	4.40	4.90	3.60	4.70
Fumonisin B3					
Trilogy	0.40	2.70	2.60	2.10	2.60

[1]A representative sample of each diet was collected from 24 bags of feed for each treatment.

[2]All mycotoxins were analyzed at NDSU (Fargo, ND) by LC/MS/MS. The standard curve for fumonisin was up to 10 ppm. Fumonisin B1, B2 and B3 were analyzed at Trilogy lab (Washington, MO) by LC/MS/MS. The standard curve for fumonisin was up to 10 ppm. Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, T-2 Toxin, ochratoxin, and sterigmatocystin were below detectable concentration (< 20 ug/kg); HT-2 toxin and vomitoxin were below detectable concentration (< 200 ug/kg); and zearalenone was below detectable concentration (< 100 ug/kg).

[3] Kallsil Dry (Kemin Industries Inc., Des Moines, IA); Feed Aid Wide Spectrum (NutriQuest, Mason City, IA); and Biofix Select Pro (Biomin America Inc., Overland Park, KS).

Table 2-3 Diet composition (as-fed basis)[1]

Item	Positive control	Negative control[2]
Ingredients, %		
Corn, 5 mg/kg of fumonisin	64.70	--
Corn, 80 mg/kg of fumonisin	--	64.48
Soybean meal	28.00	28.00
Soybean oil	3.00	3.00
Monocalcium phosphate	0.85	0.85
Calcium carbonate	0.75	0.75
Sodium chloride	0.60	0.60
L-Lysine HCl	0.55	0.55
DL-Methionine	0.21	0.21
L-Threonine	0.23	0.23
L-Tryptophan	0.06	0.06
L-Valine	0.16	0.16
Vitamin premix[3]	0.25	0.25
Trace mineral premix[3]	0.15	0.15
Phytase[4]	0.08	0.08
Sand	--	0.30
Total	100	100
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lysine	1.30	1.30
Isoleucine:lysine	53	53
Leucine:lysine	111	111
Methionine:lysine	36	36
Methionine and cysteine:lysine	56	56
Threonine:lysine	63	63
Tryptophan:lysine	20.0	20.0
Valine:lysine	69	69

Histidine:lysine	35	35
Net energy, kcal/kg	2,534	2,527
Crude protein, %	19.8	19.8
Calcium, %	0.61	0.61
STTD P[5], %	0.44	0.44

[1]Diets were fed during the 14-d treatment period. During the 13-d post treatment period, the positive control was fed to all groups of pigs.

[2]The three high fumonisin treatments containing commercial products were manufactured by using the negative control with product added at the expense of sand. Inclusion rates were 0.3% of Kallsil Dry (Kemin Industries Inc., Des Moines, IA), 0.3% of Feed Aid Wide Spectrum (NutriQuest, Mason City, IA), and 0.17% of Biofix Select Pro (Biomin America Inc., Overland Park, KS).

[3]Provided per kg complete feed: 4,133 IU vitamin A; 1653 IU vitamin D3; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg d-pantothenic acid; 8 mg riboflavin; 0.30 mg selenium; 16 mg Cu from copper sulfate; 110 mg Fe from iron sulfate; 110 mg Zn from zinc sulfate; 33 mg Mn from Manganese sulfate; 0.3 mg I from Ca iodate.

[4]Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Basel, Switzerland) provided 2,027 FTU per kg of feed and an expected STTD P release of 0.12%.

[5]STTD P = standardized total tract digestible phosphorus.

Table 2-4 Diet composition, Exp. 2 (as-fed basis)

Item	Positive control[1]	Negative control[2]
Ingredients, %		
Exp. 1 high-fumonisin diet	--	62.50
Positive control[1]	--	37.39
Corn	64.70	--
Soybean meal	28.00	--
Soybean oil	3.00	--
Monocalcium phosphate	0.85	--
Calcium carbonate	0.75	--
Sodium chloride	0.60	--
L-Lysine HCl	0.55	--
DL-Methionine	0.21	--
L-Threonine	0.23	--
L-Tryptophan	0.06	--
L-Valine	0.16	--
Vitamin premix[3]	0.25	--
Trace mineral premix[3]	0.15	--
Phytase[4]	0.08	--
Sand	--	0.11
Total	100	100
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lysine	1.30	1.30
Isoleucine:lysine	53	53
Leucine:lysine	111	111
Methionine:lysine	36	36
Methionine and cysteine:lysine	56	56
Threonine:lysine	63	63

Tryptophan:lysine	20.0	20.0
Valine:lysine	69	69
Histidine:lysine	35	35
Net energy, kcal/kg	2,534	2,527
Crude protein, %	19.8	19.8
Calcium, %	0.61	0.61
STTD P[5], %	0.44	0.44

1The positive control diet was identical to the positive control diet used in Exp. 1 and used as blending diet.

2The three high fumonisin diets with products were manufactured by using the negative control with product added at the expense of sand. 0.11% of Kallsil Dry (Kemin Industries Inc., Des Moines, IA), 0.11% of Feed Aid Wide Spectrum (NutriQuest, Mason City, IA), and 0.06% of Biofix Select Pro (Biomin America Inc., Overland Park, KS).

[3]Provided per kg complete feed: 4,133 IU vitamin A; 1653 IU vitamin D3; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg d-pantothenic acid; 8 mg riboflavin; 0.30 mg selenium; 16 mg Cu from copper sulfate; 110 mg Fe from iron sulfate; 110 mg Zn from zinc sulfate; 33 mg Mn from Manganese sulfate; 0.3 mg I from Ca iodate.

[4]Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Basel, Switzerland) provided 2,027 FTU per kg of feed and an expected STTD P release of 0.12%.

[5]STTD P = standardized total tract digestible phosphorus.

.

Table 2-5 Effect of commercial products on growth performance of nursery pigs, Exp. 1[1]

Item	Positive control	Negative control	Negative control with Kallsil Dry[2]	Negative control with Feed Aid Wide Spectrum	Negative control with Biofix Select Pro	SEM
BW, kg						
d 0	9.9	9.9	9.9	9.9	9.8	<0.17[5]
d 14	16.7[a]	13.8[b]	13.2[b]	13.6[b]	16.2[a]	0.67
d 27	57.6[a]	50.1[b]	48.0[b]	49.0[b]	56.8[a]	0.95
d 0 to 14 (treatment period)[3]						
ADG, g	483[a]	268[b]	228[b]	258[b]	454[a]	14.7
ADFI, g	717[a]	556[b]	506[b]	517[b]	690[a]	17.1
G:F, g/kg	672[a]	480[b]	448[b]	492[b]	659[a]	<25.0[6]
d 14 to 27 (post treatment period)[5]						
ADG, g	711[ab]	680[bc]	655[c]	666[bc]	736[a]	13.0
ADFI, g	1,184[a]	1,006[b]	984[b]	1,007[b]	1,170[a]	23.3
G:F, g/kg	601[c]	677[a]	668[a]	664[a]	630[b]	7.9
d 0 to 27 (overall period)						
ADG, g	592[a]	461[b]	429[b]	450[b]	590[a]	12.4
ADFI, g	940[a]	767[b]	731[b]	748[b]	921[a]	18.1
G:F, g/kg	629[ab]	601[bc]	586[c]	601[bc]	614[a]	7.2
Mortality, %	0.00	4.44	8.89	6.67	0.00	< 4.24[7]
Serum SA:SO						
d 14	0.26[b]	1.77[a]	2.15[a]	2.31[a]	1.62[a]	<0.241[8]
d 27	0.67	0.61	0.85	0.58	0.50	0.05

[a,b,c] Means within a row with different superscripts differ ($P \leq 0.05$).

[1]A total of 350 pigs (241 × 600, DNA, Columbus, NE; initially 9.9 kg) were used in a 27-d experiment with 5 pigs per pen and 14 pens per treatment. Treatment fumonisin (B1+B2, mg/kg): Positive control (4.0), Negative control (58.2), Negative + Kallsil Dry (54.1), Negative + Feed Aid Wide Spectrum (60.8) and Negative + Biofix Select Pro (51.8). BW = body weight. ADG = average daily gain. ADFI= average daily feed intake. G:F = feed efficiency. Sa = sphinganine. So = sphingosine.

[2] Kallsil Dry (Kemin Industries Inc., Des Moines, IA); Feed Aid Wide Spectrum (NutriQuest, Mason City, IA); and Biofix Select Pro (Biomin America Inc., Overland Park, KS).

[3]Except pigs in positive control group, all other pigs were fed high fumonisin diets for 14 d.

[4]All pigs were fed low fumonisin common diet for 13 d.

[5]Heterogenous SEM: Positive control (0.15), negative control (0.15), Kallsil Dry (0.15), Feed Aid Wide Spectrum (0.15) and Biofix Select Pro (0.17)

[6]Heterogenous SEM: Positive control (6.3), negative control (14.8), Kallsil Dry (14.6), Feed Aid Wide Spectrum (25.0) and Biofix Select Pro (10.9)

[7]Heterogenous SEM: Positive control (0), negative control (3.07), Kallsil Dry (4.24), Feed Aid Wide Spectrum (3.72), and Biofix Select Pro (0)

[8]Heterogenous SEM: Positive control (0.04), negative control (0.13), Kallsil Dry (0.22), Feed Aid Wide Spectrum (0.24), and Biofix Select Pro (0.20)

Table 2-6 Effect of commercial products on growth performance of nursery pigs, Exp. 2[1,2,3]

			Negative control with			
				Feed Aid		
Item	Positive control	Negative control	Kallsil Dry[2]	Wide Spectrum	Biofix Select Pro	SEM
BW, kg						
d 0	10.4	10.5	10.4	10.5	10.4	0.18
d 28	28.4[a]	25.0[b]	25.9[b]	25.4[b]	28.3[a]	0.52
ADG, g	624[a]	518[b]	546[b]	526[b]	626[a]	15.6
ADFI, g	909[ab]	844[b]	866[ab]	845[b]	934[a]	23.2
G:F, g/kg	687[a]	613[b]	631[b]	623[b]	671[a]	6.8
Mortality, %	8.15	0.00	4.01	1.99	1.99	< 4.64[3]
Serum SA:SO						
d 14	0.46[b]	1.68[a]	2.02[a]	1.36[a]	0.53[b]	<0.29[4]
d 28	0.50[b]	1.51[a]	1.46[a]	1.48[a]	0.54[b]	<0.14[5]

[a,b] Means within a row with different superscripts differ ($P \leq 0.05$).

[1]A total of 300 pigs (241 × 600, DNA, Columbus, NE; initially 10.4 kg) were used in a 28-d experiment with 5 pigs per pen and 12 pens per treatment. Treatment fumonisin (B1+B2, mg/kg): Positive control (4.7), Negative control (30.7), Negative + Kallsil Dry (31.6), Negative + Feed Aid Wide Spectrum (26.7) and Negative + Biofix Select Pro (28.5). BW = body weight. ADG = average daily gain. ADFI= average daily feed intake. G:F = feed efficiency. Sa = sphinganine. So = sphingosine.

[2] Kallsil Dry (Kemin Industries Inc., Des Moines, IA); Feed Aid Wide Spectrum (NutriQuest, Mason City, IA); and Biofix Select Pro (Biomin America Inc., Overland Park, KS).

[3]Heterogenous SEM: Positive control (4.64), negative control (0), Kallsil Dry (3.12), Feed Aid Wide Spectrum (2.11), and Biofix Select Pro (2.11)

[4]Heterogenous SEM: Positive control (0.06), negative control (0.16), Kallsil Dry (0.29), Feed Aid Wide Spectrum (0.16), and Biofix Select Pro (0.08)

[5]Heterogenous SEM: Positive control (0.06), negative control (0.14), Kallsil Dry (0.05), Feed Aid Wide Spectrum (0.12), and Biofix Select Pro (0.06)

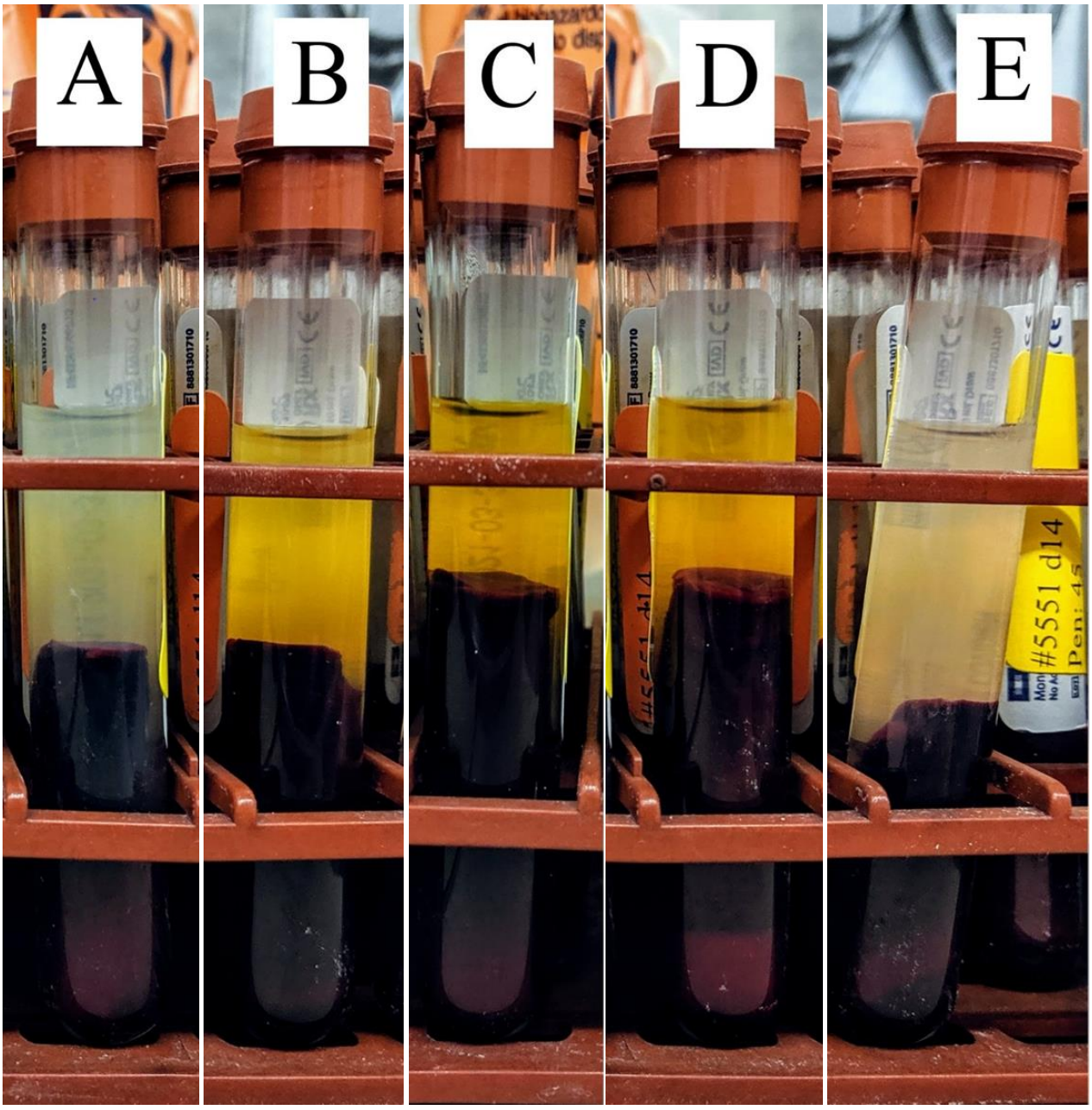

Figure 2-1. Icteric serum on d 14 of Exp. 1

A) low fumonisin positive control, B) high fumonisin negative control, C) high fumonisin diet with Kallsil Dry, D) high fumonisin diet with Feed Aid Wide Spectrum, and E) high fumonisin diet with Biofix Select Pro.

Chapter 3 - Evaluation of high-protein distillers dried grains on growth performance and carcass characteristics of growing-finishing pigs

Abstract

A total of 1,890 growing-finishing pigs (PIC; 359 × 1050; initially 27.1 kg) were used in a 124-d growth trial to compare the effects of high-protein dried distillers grains [HPDDG; 39% crude protein (CP)] or conventional dried distillers grains with solubles (DDGS; 29% CP) on growth performance and carcass characteristics. Treatments were arranged in a 2×2+1 factorial with main effects of dried distillers grains source (conventional DDGS or HPDDG) and level (15 or 30%). A corn-soybean meal-based diet served as the control and allowed linear and quadratic level effects to be determined within each dried distillers grains source. All diets were formulated on an equal SID Lys-basis with diets containing HPDDG having less soybean meal than diets with conventional DDGS. Pens were assigned to treatments in a randomized complete block design with initial weight as the blocking factor. There were 27 pigs per pen and 14 pens per treatment. Overall, increasing conventional DDGS decreased (linear, $P < 0.04$) final body weight (BW), whereas increasing HPDDG tended to decrease (linear, $P = 0.065$) final BW. The decreased final BW was a result of decreased (linear, $P < 0.01$) ADG from d 0 to 55 of the study as either DDG source increased. However, there were no differences observed in d 55 to 124 or overall ADG between pigs fed either DDG source. Pigs fed HPDDG had decreased ($P < 0.001$) ADFI and increased ($P < 0.001$) G:F compared with those fed conventional DDGS. For carcass traits, increasing either conventional DDGS or HPDDG decreased carcass yield and HCW (linear, $P < 0.02$); however, there were no differences between pigs fed HPDDG or conventional

DDGS. Iodine value (IV) increased (linear, $P < 0.02$) with increasing DDG and was greater ($P < 0.001$) in pigs fed HPDDG than conventional DDGS. In summary, pigs fed HPDDG had no evidence of difference in overall ADG compared to pigs fed conventional DDGS, but had greater overall G:F. Carcass fat IV was also greater in pigs fed HPDDG compared with pigs fed conventional DDGS. These differences were probably due to the difference in oil content.

Keywords: DDGS, high-protein distillers grains, growth, grow-finish pigs, iodine value

AA = amino acid

ADF = acid detergent fiber

ADG = average daily gain

ADFI = average daily feed intake

BCAA = branched-chain amino acids

BW = body weight

CE = caloric efficiency

CP = crude protein

DDG = dried distillers grains

DDGS = dried distillers grains with solubles

DM = dry matter

G:F = gain-to-feed ratio

HCW = hot carcass weight

HPDDG = high-protein dired distillers grains

IV = iodine value

NDF = neutral detergent fiber

NE = net energy

SBM = soybean meal

SID = standardized ileal digestible

UHPLC/MS/MS = ultra high pressure liquid chromatography and tandem mass spectrometric detection

Introduction

Distillers dried grains with solubles (**DDGS**) is a co-product of the ethanol industry that can be fed at up to 30% of the diet without negative effect for growing-finishing pigs (Stein and Shurson, 2009). Conventional DDGS has lower concentration of starch, but the concentration of oil, acid detergent fiber (**ADF**), neutral detergent fiber (**NDF**), total dietary fiber, and amino acids are greater than corn (Stein and Shurson, 2009). Nonetheless, the apparent total tract digestibility of fiber and AAs in DDGS are slightly less than in corn. The higher concentration of oil in DDGS increases the carcass fat iodine value (**IV**) which reduces the firmness of pork fat (Stein and Shurson, 2009).

New technologies have been introduced by the ethanol industry to improve the efficiency of ethanol production, resulting in new types of distiller dried grain with different nutrient profiles. One of the new processing techniques removes fibrous corn components before fermentation which results in greater ethanol production and a high-protein distillers dried grains by-product (HPDDG) with approximately 40% CP and 10% oil (Rho et al., 2017). Recent research has shown this new HPDDG product has higher CP and digestibility of some nutrients (Espinosa and Stein, 2018), and DE (Rho et al., 2017) than conventional DDGS, which may be beneficial to

growing-finishing pig performance. However, while several studies have evaluated the effect of older types of HPDDG on growing-finishing or nursery pig performance, limited research has been conducted with this new process for manufacturing HPDDG to confirm its effects on growth performance and carcass characteristics of growing-finishing pigs.

A challenge when using DDGS and HPDDG in diets for pigs is an excess dietary leucine concentration. This is due to a high level of leucine in corn protein, thus as the protein level increases in HPDDG, the leucine level increases as well. This needs to be considered because of the antagonism between different branched-chain amino acids (**BCAA**) that causes reduced growth performance (Cemin et al., 2019c). Cemin et al. (2019c) provide a basis for diet formulation to minimize BCAA antagonisms which was utilized in this study, but not in previous studies on HPDDG. Therefore, the objective of this study was to characterize the effects of this new HPDDG source on growth performance and carcass characteristics of 27 to 130-kg growing-finishing pigs when accounting for dietary BCAA levels.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research-finishing site in southwest Minnesota. The barn was naturally ventilated and double-curtain-sided. Each pen was equipped with a 5-hole stainless steel dry self-feeder and a bowl waterer for ad libitum access to feed and water.

Two groups of approximately 945 pigs (1,890 total pigs; PIC 359 × 1050; initially 27.1 ± 0.6 kg) were used in a 124-d growth trial. Pigs were housed in mixed sex pens with 27 pigs per pen and 14 pens per treatment (7 replications per group). Daily feed additions to each pen were

accomplished and recorded using a robotic feeding system (FeedPro, Feedlogic Corp., Wilmar, MN). The treatments were structured as a randomized complete block design and arranged in a 2 × 2 + 1 factorial with main effects of DDG source (conventional DDGS and HPDDG) and level (15 or 30%). A corn-soybean meal-based diet without DDG served as the control. Nutrient and SID amino acid values for DDGS were derived from NRC (2012) and nutrient and SID amino acid values for HPDDG were derived from Rho (2017). Conventional DDGS contained 29.0% CP, 9.2% oil, and an assumed 0.48% standardized ileal digestible (SID) Lys, whereas HPDDG contained 39.3% CP, 11.1% oil and an assumed 0.68% SID Lys. Conventional DDGS used in this study was made by Valero Aurora Ethanol Plant (Aurora, SD). High-protein DDG used in this study was made by ICM Biofuels (St Joseph, MO). Corn, conventional DDGS, and HPDDG used in this trial were analyzed for proximate analysis, amino acid profile (Table 1) and mycotoxins (Table 2). The multiple mycotoxin assay conducted at North Dakota State University Veterinary Diagnostic Laboratory was based on an Agilent Technologies (Santa Clara, CA) method for mycotoxin in corn using ultra high pressure liquid chromatography and tandem mass spectrometric detection (**UHPLC/MS/MS**) with modifications (Varga et al., 2013). The detail of this analysis can be found in Rao et al. (2020).

Dietary treatments were fed in 4 phases based on body weight. All diets were formulated on an equal SID Lys-basis with diets containing HPDDG having less soybean meal (**SBM**) than diets with conventional DDGS and the control diet (Table 3 and 4). By design, NE was not balanced between treatments for each phase. Thus, differences in feed efficiency would reflect differences in energy value of the DDG source. In addition, dietary BCAA ratios were adjusted based on the equation of Cemin et al. (2019a) to account for the excess dietary leucine in the conventional DDGS and HPDDG. We increased our formulated dietary SID Ile:Lys and Val:Lys

ratios as the level of conventional DDGS or HPDDG increased. Because of the greater Ile, Leu and Val concentration in HPDDG than conventional DDGS, diets with HPDDG had greater BCAA:Lys ratios compared to conventional DDGS at the same dietary inclusion (15 or 30%) This was also a result of the higher CP level of HPDDG that resulted in lower SBM inclusion level compared to diet with DDGS (Table 3 and 4).

Pigs were weighed approximately every 14 days from d 0 to 124 of the trial to determine ADG, ADFI, and G:F. Caloric efficiency (CE) was calculated to determine the energy estimate of HPDDG. Caloric efficiency has been used to estimate the energy of an ingredient by comparing it to a known ingredient, such as corn (Cemin et al., 2020). By increasing the inclusion level of the tested ingredient in diet, CE should remain the same if the energy estimation is accurate.

On d 103, the 3 heaviest pigs in each pen were selected and marketed as per the standard farm marketing protocol. These pigs were included in the growth performance data but not in carcass data. On the last day of the trial, final pen weights were taken, and the remaining pigs were tattooed with a pen identification number and transported to a USDA-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (**HCW**), loin depth, backfat, percentage lean, and fat iodine value (**IV**). Fat samples for IV analysis were collected from the shoulder of carcasses with 14 pigs per treatment. The fat samples were analyzed using Fourier transform near infrared (FT-NIR) on a Bruker MATRIX-I FT-NIR spectrometer (Billerica, MA). Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by taking the pen average HCW divided by the pen average final live weight obtained at the farm.

Statistical analysis

Data were analyzed as a randomized complete block design for two-way ANOVA using the lmer function from the lme4 package in R program (R Core Team, 2019) with pen considered the experimental unit, initial BW as blocking factor, and treatment as fixed effect. Phase 1 and 2 were combined to represent the grower phase, while phase 3 and 4 were combined and referred to as the finisher phase for growth performance analysis. Predetermined contrasts were used to evaluate the main effects and interactive effects of DDG source × level among treatments. Preplanned contrasts were also used to examine the linear and quadratic responses due to increasing DDG addition within DDG source using the control diet (as 0% inclusion level) and the 15% and 30% diets. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Based on the literature from 2007 to 2018 and NRC (2012), there are several types of HPDDG with or without solubles (Widmer et al., 2007; 2008; Kim et al., 2009; Jacela et al., 2010; Almeida and Stein, 2012; Gutierrez et al., 2014; Petersen et al., 2014; Adeola and Ragland, 2016; Rho et al., 2017; Espinosa and Stein, 2018; Yang et al., 2018). According to the nutrient analysis, these HPDDG sources have similar AA to CP ratios, but CP, Ca, P, oil, and NDF content varies. The major difference between the other HPDDG sources and the HPDDG used in the current study is that before fermentation of the corn starch to ethanol the fiber component of the corn kernel is removed (M. Wilken, personal communication). This processing method reduces the ADF content of HPDDG with NDF remaining relatively unchanged as a percentage of product compares to conventional DDGS. This results in a HPDDG source that has

a greater oil content (9.24 vs. 3.38%) compared to other HPDDG sources on the market. The variation in nutrient composition of HPDDG can be a result of differences in processing methods, the type of yeast used for fermentation, the complexity of dry-grind process, and the amount of solubles added back (Liu, 2011).

The HPDDG used in this trial had a greater crude protein (39.2 vs. 29.0%) and oil content (10.3 vs. 8.0%) compared to the conventional DDGS. Furthermore, this HPDDG had higher oil content (10.3 vs. 3.54%), Ca (0.17 vs. 0.02), P (0.73 vs. 0.36%), and lower CP (39.2 vs. 45.4%) compared to the values listed for HPDDG in NRC (2012), therefore caution should be used when applying nutrient values from NRC (2012) for HPDDG because of the many manufacturing processes. The total P content was lower in HPDDG compared to conventional DDGS (0.73 vs. 1.08%) because HPDDG did not contain the soluble portion of distillers dried grain production. The ratios of AAs to CP were similar between corn, HPDDG, and conventional DDGS, however the digestibility of AAs may be lower for DDGS and HPDDG compared to corn because of the DDG production processes (Stein and Shurson, 2009). Rho et al. (2017) compared similar HPDDG as in the current experiment with conventional DDGS and found that DE and SID amino acid values of indispensable AAs and CP were greater compared to conventional DDGS. Espinosa et al. (2018) observed that similar HPDDG used in growing-finishing pigs had greater ($P < 0.05$) apparent total tract digestibility (**ATTD**) of DE and ME, SID Leu, Lys, Met, Phe, and Glu compared to conventional DDGS. These results suggest that this HPDDG has greater nutritional value than conventional DDGS.

Three batches of corn, HPDDG, and conventional DDGS were used and analyzed for mycotoxins (North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND). Fumonisin concentrations ranged from approximately 8 to 15 mg/kg in HPDDG and 0.2 to 0.3

mg/kg in conventional DDGS (Table 2). Vomitoxin concentrations were similar between DDG sources which ranged from approximately 0.8 to 1.0 mg/kg. Based on the inclusion of the DDG sources, these mycotoxin levels would not be considered to negatively affect growing-finishing pig health and performance (Ensely and Radke, 2019; Rao et al., 2020). Chemical analysis (Table 3 and 4) of treatment diets for dry matter, crude protein, Ca, P, neutral detergent fiber, and ether extract were within formulated ranges.

In the grower phase (day 0 to 55), increasing either DDG source decreased (linear, $P < 0.001$) ADG (Table 5). The addition of DDGS did not influence ADG in the finishing phase (day 55 to 124), except for a tendency for pigs fed 15% conventional DDGS to have lower (quadratic, $P = 0.065$) ADG compared to those fed 0 or 30%. There was no evidence of differences observed in overall ADG. Despite no overall changes in ADG between treatments, increasing conventional DDGS decreased (linear, $P = 0.039$) final body weight (BW), whereas increasing HPDDG tended to decrease (linear, $P = 0.065$) final BW. This is likely due to changes in ADG during the grower phase. Increasing HPDDG decreased (linear, $P \leq 0.002$) ADFI and improved (linear, $P \leq 0.002$) G:F in both phases and the overall period, whereas there was no change in ADFI or G:F among pigs fed conventional DDGS.

Conventional DDGS and HPDDG have higher SID Ile:Lys, Leu:Lys, and Val:Lys ratios and lower Lys:CP ratio compared to SBM. Therefore, including these ethanol co-products and reducing SBM inclusion to achieve the same SID Lys would increase the SID Ile:Lys, Leu:Lys, and Val:Lys ratios in the diets. Isoleucine, Leu, and Val are branched-chain amino acids (BCAA) that have similar molecular structure and share several catabolism pathways (Harper et al., 1984). All BCAAs can be catabolized by branched-chain aminotransferase and branched-chain α-keto acid dehydrogenase complex. Excess of either of the BCAA in diets upregulate the

enzymatic activity which would increase the catabolism of all BCAA. This antagonistic effect reduces the growth performance of growing-finishing pigs (Cemin et al., 2019c). Therefore, we formulated our diets based on the equation developed by Cemin et al. (2019a) to predict equal ADG by adjusting dietary SID Ile:Lys, Leu:Lys, Trp:Lys, and Val:Lys ratios accordingly.

Based on the improved G:F and decreased ADFI in pigs fed HPDDG, its energy content appears to be greater than the conventional DDGS used in this study. The improvement in G:F of pigs fed increasing HPDDG compared to conventional DDGS may be due to the higher oil content or improved nutrient digestibility in HPDDG. By calculating the caloric efficiency (**CE**) of diets using procedures of Cemin et al. (2019b), CE was linearly improved ($P = 0.033$) as HPDDG increased. Therefore, we suspected that the net energy (**NE**) of HPDDG was underestimated. For CE of HPDDG diets to be identical to the control diet, the NE of HPDDG would have to be 103.4% of the energy of corn, which was greater than the value (97.3%; Cemin et al., 2019a) which was used for diet formulation.

Yang et al. (2018) fed 0 to 30% of HPDDG from similar processing method as that used in this study to nursery pigs and found that increasing HPDDG linearly decreased ($P < 0.01$) ADG, ADFI and G:F. The difference in ADG response in their experiment compared to ours may be due to the approach in diet formulation. Applying their dietary AA values to the equation of Cemin et al. (2019a) results in a predicted reduction in ADG as the inclusion of HPDDG increases. This is because of the high SID Leu:Lys ratio and relatively low Val:Leu and Ile:Leu ratios. Also, the SID AA coefficients used in their trial were determined in 75-kg growing-finishing pig (Espinosa and Stein, 2018), which may lead to overestimation of AA digestibility for nursery pigs (Nitrayová et al., 2006). Furthermore, the high fiber content in diets containing HPDDG may cause reduced feed intake because of the increased bulk volume of feed in the

intestine (Nyachoti et al., 2004; Avelar et al., 2010). These results suggest the importance of adjusting dietary BCAA levels to account for the high leucine content of HPDDG, which allow producers to fully obtain the benefit of HPDDG and mitigate the negative effects.

For carcass characteristics, increasing either conventional DDGS or HPDDG decreased carcass yield and HCW (linear, $P < 0.02$). This was expected as conventional DDGS and HPDDG increase the dietary crude fiber content of the diets which increased gut fill and intestine mass of pigs (Stein and Shurson, 2009). Because intestine weight is not included in carcass weight, the carcass yield and HCW were reduced. There were no differences among dietary treatments in back fat, loin depth or percentage lean.

Carcass fat iodine value (IV) is an important measure for carcass fat softness. High IV results in low belly fat firmness which is undesirable (Stein and Shurson, 2009). Carcass fat IV was greater ($P < 0.001$) in pigs fed HPDDG than conventional DDGS, and IV increased (linear, $P < 0.02$) with increasing inclusion level of either DDG source. Similar to the improvements in G:F, the change in IV between pigs fed HPDDG and conventional DDGS was most likely due to the differences in oil content.

In summary, these data suggest that feeding pigs up to 30% of the diet of HPDDG may have economic advantages because of its amino acid profile and improved G:F compared with those fed conventional DDGS. However, caution must be used with the type of HPDDG used because of the different nutrient profiles, especially AA profile, oil and energy content. Accurate AA profile allows adjustment of SID Ile:Lys, Leu:Lys, Trp:Lys, and Val:Lys to avoid BCAA imbalance that may cause reduced growth performance. A potential concern with many DDG sources is the oil content which if high enough, leads to high unsaturated fat in the carcass and increased carcass fat IV. Therefore, applying conventional DDGS or HPDDG in growing-

finishing pig diets has the potential to lower diet cost while maintaining similar ADG and G:F to a corn-soybean meal based diet. However, when using DDG sources, dietary branched-chain amino acid ratios should be accounted for to maintain growth performance. Dietary oil content should be considered to avoid reduced carcass fat firmness and high carcass fat IV.

References

Adeola, O., and D. Ragland. 2016. Comparative ileal amino acid digestibility of distillers' grains for growing pigs. Anim. Nutr. 2(4):262-266. doi: https://doi.org/10.1016/j.aninu.2016.07.008

Almeida, F. N., and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs1. J. Anim. Sci. 90(4):1262-1269. doi: 10.2527/jas.2011-4144

Avelar, E., R. Jha, E. Beltranena, M. Cervantes, A. Morales, and R. T. Zijlstra. 2010. The effect of feeding wheat distillers dried grain with solubles on growth performance and nutrient digestibility in weaned pigs. Anim. Feed Sci. Technol. 160(1):73-77. doi: https://doi.org/10.1016/j.anifeedsci.2010.06.009

Cemin, H. S., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, and R. D. Goodband. 2019a. Meta-regression analysis to predict the influence of branched-chain and large neutral amino acids on growth performance of pigs1. J. Anim. Sci. 97(6):2505-2514. doi: 10.1093/jas/skz118

Cemin, H. S., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, and R. D. Goodband. 2019b. PSVI-20 Evaluating the productive energy content of high-protein distillers dried grains in swine diets. J. Anim. Sci. 97(Supplement_2):211-212. doi: 10.1093/jas/skz122.371

Cemin, H. S., M. D. Tokach, J. C. Woodworth, S. S. Dritz, J. M. DeRouchey, and R. D. Goodband. 2019c. Branched-chain amino acid interactions in growing pig diets. Transl. Anim. Sci. 3(4):1246-1253. doi: 10.1093/tas/txz087

Cemin, H. S., H. E. Williams, M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, K. F. Coble, B. A. Carrender, and M. J. Gerhart. 2020. Estimate of the energy value of soybean meal relative to corn based on growth performance of nursery pigs. J. Anim. Sci. Biotechnol. 11(1):70. doi: 10.1186/s40104-020-00474-x

Ensely, S. M., and S. L. Radke. 2019. Mycotoxins in Grains and Feeds, Diseases of Swine. Wiley-Blackwell, Hoboken, NJ. p. 1055-1071.

Espinosa, C. D., and H. H. Stein. 2018. High-protein distillers dried grains with solubles produced using a novel front-end-back-end fractionation technology has greater nutritional value than conventional distillers dried grains with solubles when fed to growing pigs. J. Anim. Sci. 96(5):1869-1876. doi: 10.1093/jas/sky052

Gutierrez, N. A., D. Y. Kil, Y. Liu, J. E. Pettigrew, and H. H. Stein. 2014. Effects of co-products from the corn-ethanol industry on body composition, retention of protein, lipids and energy, and on the net energy of diets fed to growing or finishing pigs. J. Sci. Food Agric. 94(14):3008-3016. doi: 10.1002/jsfa.6648

Harper, A. E., R. H. Miller, and K. P. Block. 1984. Branched-chain amino acid metabolism. Annu. Rev. Nutr. 4:409-454. doi: 10.1146/annurev.nu.04.070184.002205

Jacela, J. Y., H. L. Frobose, J. M. DeRouchey, M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. L. Nelssen. 2010. Amino acid digestibility and energy concentration of high-protein corn dried distillers grains and high-protein sorghum dried distillers grains with solubles for swine12. J. Anim. Sci. 88(11):3617-3623. doi: 10.2527/jas.2010-3098

Kim, B. G., G. I. Petersen, R. B. Hinson, G. L. Allee, and H. H. Stein. 2009. Amino acid digestibility and energy concentration in a novel source of high-protein distillers dried

grains and their effects on growth performance of pigs. J. Anim. Sci. 87(12):4013-4021. doi: 10.2527/jas.2009-2060

Liu, K. 2011. Chemical composition of distillers grains, a review. J. Agric. Food. Chem. 59(5):1508-1526. doi: 10.1021/jf103512z

Nitrayová, S., J. Heger, P. Patras, and M. Brestenský. 2006. Effect of body weight and pig individuality on apparent ileal digestibility of amino acids and total nitrogen. Slovak J. Anim. Sci 39:65-68.

Nyachoti, C. M., R. T. Zijlstra, C. F. M. de Lange, and J. F. Patience. 2004. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 84(4):549-566. doi: 10.4141/A04-001

Petersen, G. I., Y. Liu, and H. H. Stein. 2014. Coefficient of standardized ileal digestibility of amino acids in corn, soybean meal, corn gluten meal, high-protein distillers dried grains, and field peas fed to weanling pigs. Anim. Feed Sci. Technol. 188:145-149. doi: https://doi.org/10.1016/j.anifeedsci.2013.11.002

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing

Rao, Z. X., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, H. Calderon Cartagena, and S. S. Dritz. 2020. Effects of fumonisin-contaminated corn on growth performance of 9- to 28-kg nursery pigs: in press. Master thesis, Kansas State University, Manhattan, KS.

Rho, Y., C. Zhu, E. Kiarie, and C. F. M. de Lange. 2017. Standardized ileal digestible amino acids and digestible energy contents in high-protein distiller's dried grains with solubles fed to growing pigs. J. Anim. Sci. 95(8):3591-3597. doi: 10.2527/jas.2017.1553

Stein, H. H., and G. C. Shurson. 2009. BOARD-INVITED REVIEW: The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci. 87(4):1292-1303. doi: 10.2527/jas.2008-1290

Varga, E., K. Mayer, M. Sulyok, R. Schuhmacher, R. Krska, and F. Berthiller. 2013. Validation of a Stable Isotope Dilution Assay for the Accurate Quantitation of Mycotoxin in Maize Using UHPLC/MS/MS. Agilent Technologies, Inc., Waldbronn, Germany.

Widmer, M. R., L. M. McGinnis, and H. H. Stein. 2007. Energy, phosphorus, and amino acid digestibility of high-protein distillers dried grains and corn germ fed to growing pigs1. J. Anim. Sci. 85(11):2994-3003. doi: 10.2527/jas.2006-840

Widmer, M. R., L. M. McGinnis, D. M. Wulf, and H. H. Stein. 2008. Effects of feeding distillers dried grains with solubles, high-protein distillers dried grains, and corn germ to growing-finishing pigs on pig performance, carcass quality, and the palatability of pork. J. Anim. Sci. 86(8):1819-1831. doi: 10.2527/jas.2007-0594

Yang, Z., P. E. Urriola, A. M. Hilbrands, L. J. Johnston, and G. C. Shurson. 2018. Growth performance of nursery pigs fed diets containing increasing levels of a novel high-protein corn distillers dried grains with solubles1. Transl. Anim. Sci. 3(1):350-358. doi: 10.1093/tas/txy101

Table 3-1 Chemical analysis of ingredients (as-fed basis)[1,2]

Item	Corn	Conventional DDGS	HPDDG
Proximate analysis, %			
Dry matter	86.53	90.15	92.62
Crude protein	6.73	29.03	39.17
Calcium	0.10	0.12	0.17
Phosphorus	0.33	1.08	0.73
Neutral detergent fiber	6.83	29.87	30.60
Ether extract	3.20	8.03	10.27
Indispensable amino acids, %			
Arginine	0.26	1.33	1.77
Histidine	0.18	0.84	1.08
Isoleucine	0.22	1.17	1.64
Leucine	0.69	3.34	4.69
Lysine	0.22	1.03	1.48
Methionine	0.12	0.51	0.82
Phenylalanine	0.29	1.50	2.08
Threonine	0.22	1.13	1.51
Tryptophan	0.05	0.22	0.33
Valine	0.29	1.48	2.06
Dispensable amino acids, %			
Alanine	0.43	1.90	2.73
Aspartic Acid	0.42	1.79	2.63
Cysteine	0.14	0.58	0.76
Glutamic Acid	1.06	3.53	5.79
Glycine	0.24	1.18	1.51
Proline	0.52	2.21	3.01
Serine	0.27	1.27	1.69
Taurine	0.10	0.07	0.08
Tyrosine	0.13	1.04	1.46

[1]Representative samples of each batch of each ingredient was collected, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and amino acid analysis (Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO). The result shown was the average of three batches of ingredients used.

[2]DDGS = dried distillers grain with solubles. HPDDG = high protein dried distillers grain.

Table 3-2 Dietary mycotoxin concentrations (as-fed basis, ug/kg)[1]

Item	Corn	Conventional DDGS[2]	HPDDG
Aflatoxin B1	< 20	< 20	< 20
Aflatoxin B2	< 20	< 20	< 20
Aflatoxin G1	< 20	< 20	< 20
Aflatoxin G2	< 20	< 20	< 20
Fumonisin B1	< 200	240.0	9234.3
Fumonisin B2	< 200	< 200	2949.7
HT-2 toxin	< 200	< 200	< 200
T-2 Toxin	< 20	< 20	< 20
Ochratoxin	< 20	< 20	< 20
Sterigmatocystin	< 20	< 20	< 20
Zearalenone	< 100	122.3	305.7
Vomitoxin	418.3	897.7	868.3

[1]Representative samples of each ingredient were collected for each batch. The result was reported as the average of three batches. Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay.

[2]DDGS = dried distillers grain with solubles. HPDDG = high protein dried distillers grain.

Table 3-3 Composition of phase 1 and 2 diets (as-fed basis)[1, 2]

Items	Phase 1					Phase 2				
	Control	Conventional DDGS[3]		HPDDG		Control	Conventional DDGS		HPDDG	
		15%	30%	15%	30%		15%	30%	15%	30%
Ingredients, %										
Corn	74.30	61.92	50.42	63.24	52.93	80.26	69.06	57.56	70.31	60.08
Soybean meal	22.52	20.14	16.82	18.94	14.42	16.91	13.27	9.94	12.07	7.54
Corn DDGS	--	15.00	30.00	--	--	--	15.00	30.00	--	--
HPDDG	--	--	--	15.00	30.00	--	--	--	15.00	30.00
Limestone, ground	1.00	1.13	1.23	1.10	1.20	0.95	1.05	1.15	1.05	1.13
Monocalcium phosphate	0.65	0.40	0.15	0.40	0.15	0.50	0.25	0.00	0.30	0.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.48	0.50	0.55	0.50	0.55	0.44	0.50	0.55	0.50	0.55
DL-Methionine	0.14	0.08	0.03	0.02	0.00	0.10	0.05	0.00	0.00	0.00
L-Threonine	0.23	0.19	0.15	0.17	0.11	0.19	0.17	0.13	0.14	0.08
L-Tryptophan	0.05	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.05	0.04
L-Valine	0.11	0.08	0.08	0.07	0.08	0.09	0.09	0.10	0.06	0.07
Vitamin and trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[5]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis										
Standardized ileal digestible (SID) amino acids, %										
Lysine	1.13	1.13	1.13	1.13	1.13	0.96	0.96	0.96	0.96	0.96
Isoleucine:lysine	55	59	62	63	70	55	57	60	62	69
Leucine:lysine	113	134	153	151	186	119	141	163	161	203
Methionine:lysine	34	32	30	29	33	33	31	30	29	36
Methionine and cysteine:lysine	56	56	56	56	65	56	56	57	58	70
Threonine:lysine	65	65	65	65	65	65	65	65	65	65
Tryptophan:lysine	19.0	18.8	19.1	18.8	19.1	18.8	18.9	18.8	18.9	18.7
Valine:lysine	70	74	79	78	88	70	76	82	78	90
Lysine:net energy, g/Mcal	4.42	4.49	4.55	4.39	4.35	3.69	3.74	3.79	3.66	3.63
Net energy, kcal/kg	2,552	2,510	2,477	2,567	2,589	2,594	2,563	2,530	2,616	2,642
STTD P, %	0.38	0.38	0.38	0.38	0.38	0.34	0.34	0.34	0.34	0.33
Proximate analysis, %[6]										

Dry matter	87.02	88.69	88.63	88.49	89.22	88.09	88.06	89.84	88.31	89.35
Crude protein	14.9	17.2	19.7	18.1	22.0	16.1	16.9	17.5	17.7	20.4
Calcium	0.60	0.53	0.53	0.52	0.48	0.58	0.50	0.53	0.48	0.45
Phosphorus	0.41	0.44	0.46	0.40	0.41	0.42	0.41	0.44	0.39	0.37
Neutral detergent fiber	7.2	10.4	13.8	9.2	14.2	7.6	10.6	12.8	9.7	13.8
Ether extract	2.9	3.9	4.5	4.3	5.6	3.1	3.9	4.9	4.4	5.4

[1]Phases 1 and 2 were fed from 27 to 50 and 50 to 73 kg, respectively.

[2]Equation used for ADG (g/d) prediction (Cemin et al., 2019a): – 985.94 + (15.2499 × average BW (kg)) – (0.08885 × average BW × average BW) + (1.063 × Leu:Lys) + (20.2659 × Ile:Lys) – (0.1479 × Ile:Lys × Ile:Lys) + (9.2243 × (Ile+Val):Leu) – (0.03321 × (Ile+Val):Leu × (Ile+Val):Leu) – (0.4413 × Ile:Trp)

[3]DDGS = dried distillers grain with solubles. HPDDG = high protein dried distillers grain.

[4]Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; and 0.20 g Se from sodium selenite.

[5]Optiphos 2000 (Huvepharma Inc. Peachtree City, GA) provided 871 units of phytase FTU/kg of diet with an assumed release of 0.11% STTD P.

[6]At least 6 representative samples of each diet were collected for each treatment, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE).

Table 3-4 Composition of phase 3 and 4 diets (as-fed basis)[1, 2]

Items	Phase 3					Phase 4				
	Control	Conventional DDGS[3]		HPDDG		Control	Conventional DDGS		HPDDG	
		15%	30%	15%	30%		15%	30%	15%	30%
Ingredients, %										
Corn	84.75	73.32	61.78	74.52	64.28	86.15	75.37	63.83	76.64	66.33
Soybean meal	12.52	9.19	5.87	8.00	3.47	11.29	7.17	3.85	5.97	1.45
Corn DDGS	--	15.00	30.00	--	--	--	15.00	30.00	--	--
HPDDG	--	--	--	15.00	30.00	--	--	--	15.00	30.00
Limestone, ground	0.93	1.03	1.13	1.03	1.10	0.90	1.00	1.10	1.00	1.10
Monocalcium phosphate	0.55	0.25	0.00	0.30	0.00	0.50	0.25	0.00	0.25	0.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.40	0.45	0.50	0.45	0.50	0.38	0.45	0.50	0.45	0.50
DL-Methionine	0.06	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00
L-Threonine	0.16	0.13	0.09	0.11	0.05	0.15	0.12	0.09	0.10	0.04
L-Tryptophan	0.05	0.05	0.05	0.04	0.04	0.04	0.05	0.05	0.04	0.04
L-Valine	0.06	0.07	0.06	0.04	0.04	0.04	0.07	0.06	0.03	0.02
Vitamin and trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[5]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis										
Standardized ileal digestible (SID) amino acids, %										
Lysine	0.82	0.82	0.82	0.82	0.82	0.77	0.77	0.77	0.77	0.77
Isoleucine:lysine	55	58	61	63	72	55	57	60	63	72
Leucine:lysine	127	153	179	176	225	132	157	185	182	234
Methionine:lysine	32	29	33	32	39	31	29	33	33	41
Methionine and cysteine:lysine	56	56	62	63	78	56	57	64	65	80
Threonine:lysine	65	65	65	65	65	65	65	65	65	65
Tryptophan:lysine	19.1	19.0	18.8	18.9	18.7	18.9	18.8	18.6	18.8	18.6
Valine:lysine	70	77	83	80	94	70	78	84	80	93
Lysine:net energy, g/Mcal	3.12	3.16	3.20	3.09	3.07	2.92	2.95	2.99	2.89	2.86
Net energy, kcal/kg	2,622	2,589	2,556	2,644	2,669	2,631	2,605	2,572	2,660	2,682
STTD P, %	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.32	0.32	0.32
Proximate analysis, %[6]										

Dry matter	86.95	87.91	88.25	88.28	88.53	87.94	88.63	89.49	88.07	88.87
Crude protein	12.0	13.2	15.7	15.1	18.3	12.5	14.2	14.6	15.1	17.2
Calcium	0.57	0.58	0.45	0.51	0.58	0.55	0.49	0.45	0.55	0.50
Phosphorus	0.37	0.40	0.43	0.36	0.35	0.40	0.40	0.41	0.36	0.32
Neutral detergent fiber	7.8	9.0	13.3	10.5	12.7	7.7	10.9	12.1	10.5	12.6
Ether extract	3.5	3.9	4.8	4.5	5.5	3.5	4.4	4.7	4.7	5.4

[1]Phases 3 and 4 were fed from 73 to 100 and 100 kg to marketing, respectively.

[2]Equation used for ADG (g/d) prediction (Cemin et al., 2019a): – 985.94 + (15.2499 × average BW (kg)) – (0.08885 × average BW × average BW) + (1.063 × Leu:Lys) + (20.2659 × Ile:Lys) – (0.1479 × Ile:Lys × Ile:Lys) + (9.2243 × (Ile+Val):Leu) – (0.03321 × (Ile+Val):Leu × (Ile+Val):Leu) – (0.4413 × Ile:Trp)

[3]DDGS = dried distillers grain with solubles. HPDDG = high protein dried distillers grain.

[4]Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; and 0.20 g Se from sodium selenite.

[5]Optiphos 2000 (Huvepharma Inc. Peachtree City, GA) provided 871 units of phytase FTU/kg of diet with an assumed release of 0.11% STTD P.

[6]At least 6 representative samples of each diet were collected for each treatment, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE).

Table 3-5 The effects of DDG source and level on grow-finish pig growth performance, and carcass characteristics[1, 2]

							Probability, *P* =				
		Conventional DDGS		HPDDG				Conventional DDGS		HPDDG	
Item[2]	Control (0%)	15%	30%	15%	30%	SEM	Source	Linear	Quadratic	Linear	Quadratic
BW, kg											
Initial	27.1	27.1	27.1	27.1	27.1	0.61	0.853	0.801	0.955	0.948	0.940
Ending	130.0	127.3	127.8	129.0	128.0	2.29	0.199	0.039	0.098	0.065	0.974
Grower phase[3]											
ADG, g	893	879	862	875	852	15.8	0.249	< 0.001	0.831	< 0.001	0.772
ADFI, g[5]	1,870	1,840	1,828	1,825	1,721	40.7	0.002	0.121	0.696	< 0.001	0.197
G:F, g/kg[5]	479	479	472	480	497	4.00	< 0.001	0.180	0.380	< 0.001	0.081
CE, kcal/kg	5,395	5,317	5,322	5,420	5,300	44.7	0.271	0.164	0.353	0.071	0.109
Finisher phase[4]											
ADG, g	855	833	864	860	870	20.3	0.166	0.571	0.065	0.368	0.858
ADFI, g[6]	2,609	2,604	2,644	2,555	2,510	54.8	< 0.001	0.256	0.399	0.002	0.862
G:F, g/kg	328	321	327	336	347	4.67	< 0.001	0.868	0.160	0.001	0.840
CE, kcal/kg	8,040	8,136	7,890	7,937	7,743	107.6	0.064	0.250	0.132	0.026	0.689
Overall											
ADG, g	876	857	865	870	863	6.8	0.375	0.252	0.101	0.194	0.950
ADFI, g[5]	2,262	2,243	2,259	2,212	2,139	30	< 0.001	0.902	0.422	< 0.001	0.603
G:F, g/kg	388	382	384	394	404	4.15	< 0.001	0.371	0.393	< 0.001	0.592
CE, kcal/kg	6,747	6,758	6,655	6,699	6,586	71.6	0.225	0.220	0.380	0.033	0.606
Carcass characteristics											
HCW, kg	94.9	92.5	92.1	94.0	92.0	1.50	0.189	< 0.001	0.127	< 0.001	0.443
Carcass yield, %	73.1	72.6	72.1	72.9	71.9	0.324	0.920	0.019	0.849	0.005	0.231
Backfat depth, mm[7]	15.9	15.5	15.9	15.8	15.6	0.28	0.978	0.954	0.241	0.421	0.699
Loin depth, mm[7]	67.0	67.0	66.9	67.3	66.7	0.46	0.828	0.847	0.947	0.684	0.426
Lean, %[7]	57.2	57.5	57.2	57.3	57.4	0.181	0.978	0.901	0.272	0.552	0.909
Iodine value[6], g/100g	64.8	69.0	73.7	72.9	80.0	0.76	< 0.001	< 0.001	0.818	< 0.001	0.546

[1]A total of 1,890 pigs (initially 27.1 kg) were used in two groups with 27 pigs per pen and 14 replicates per treatment.

[2]DDGS = dried distillers grain with solubles. HPDDG = high protein dried distillers grain. BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency (the amount of energy consumed per kg of BW gain). HCW = hot carcass weight.

[3]Grower phase was from d 0 to 55 in group 1 and from d 0 to 55 in group 2.

[4]Finisher phase was from d 55 to 113 in group 1 and from d 55 to 124 in group 2.

[5]Interactive effect, source × level $P \leq 0.05$.

[6]Interactive effect, source × level $0.05 < P \leq 0.10$.

[7]Adjusted using HCW as covariate.