Evaluation of strategies to improve efficiency in swine production and minimize pathogen transmission through feed

by

Jordan Thomas Gebhardt

B.S., Michigan State University, 2014
D.V.M., Kansas State University, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
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Abstract

Efficient use of resources is an important goal of modern agriculture. Several approaches to maximize resource utilization in swine production were evaluated including dietary approaches and interventions within the feed manufacturing process to optimize animal health. A total of 7,842 pigs were used over a total of 10 experiments structured in 6 chapters. Chapter 1 evaluated the effects of roller mill configuration on growth performance of nursery and finishing pigs, feed preference, and feed mill throughput. The four experimental treatments included corn ground through a roller mill using two, three, four sets of rolls in a fine-grind configuration, or four sets of rolls in a coarse grind configuration. There was no evidence of differences observed for average daily gain (ADG) or average daily feed intake (ADFI) between roller mill configurations when fed to nursery pigs. However, when given a choice nursery pigs consumed more of the diet containing corn ground through the 2-high roller mill or 4-high coarse configuration compared to corn ground through the 4-high fine configuration. Finally, finishing pigs fed corn ground with the 2-high configuration had greater ADG compared to those fed corn ground using the 3-high configuration. Grinding rate was greatest for the 4-high coarse configuration, while net electricity consumption was lowest for the 2-high configuration and greatest for the 4-high fine configuration. Chapter 2 evaluated the impact of commercial feed additives on the quantification of genetic material and infectivity of swine feed inoculated with porcine epidemic diarrhea virus (PEDV). The combination of essential oils and benzoic acid enhanced degradation of PEDV ribonucleic acid (RNA) in feed but had little impact in spray-dried porcine plasma. In addition, differences in viral stability was observed between feed and spray-dried porcine plasma where PEDV could be detected and remained infectious longer compared to swine feed. Chapter 3 evaluated the impact of flushing feed manufacturing

equipment with rice hull flushes following mixing PEDV infected feed. Flushing effectively reduced the quantity of detectible RNA present after mixing a batch of PEDV-positive feed. Furthermore, chemical treatment of rice hulls with formaldehyde or 10% medium chain fatty acid (MCFA) provided additional reduction in RNA detection. Chapters 4 and 5 evaluated the inclusion of added chromium (Cr) in finishing diets. In chapter 4, small differences in ADG and feed efficiency were observed with added Cr. In chapter 5, adding Cr along with *Yucca schidigera* led to modest changes in performance with the greatest benefit observed with 200 µg/kg Cr and 125 mg/kg *Yucca schidigera*-based feed grade concentrate. Chapter 6 evaluated the impact of feeding MCFA to nursery pigs and demonstrated improved growth performance, but did not significantly alter fecal microbial composition, and provided residual mitigation activity when inoculated with PEDV following feed storage.

Overall, evaluation of feed manufacturing technologies and various feed additives demonstrates potential to have a significant impact on the efficiency of swine production. Additionally, understanding the role that feed and feed transportation contributes to health of swine populations is critical for maintaining a high health and productive global swine industry.

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I would like to express many thanks to my family (Mom, Dad, Jake, Paige) who emphasized the value of hard work and moral character which has shaped my upbringing and development both personally and professionally. Finally, I would like to express many thanks to my peers both past and present who have provided support, encouragement, and of course physical labor. Without the cooperative effort of all, none of this would be possible. Thank you all. Go Cats!

Preface

This dissertation is original work completed by the author, J. T. Gebhardt. Chapters 1 (doi:10.1093/jas/sky147), 3 (doi:10.1093/jas/sky295), and 6 (doi:10.1093/jas/skz358) were published in Journal of Animal Science. Chapters 2 (doi:10.1093/tas/txy100), 4 (doi:10.1093/tas/txy104), and 5 (doi:10.1093/tas/txz117) were published in Translational Animal Science. Each of the chapters was formatted according to the required standards of the corresponding journal.

Chapter 1 - Effect of roller mill configuration on growth performance of nursery and finishing pigs and milling characteristics[1]

ABSTRACT

Three experiments were conducted to evaluate the effects of roller mill configuration on growth performance of nursery and finishing pigs, feed preference, and feed mill throughput. The 4 experimental treatments included corn ground through a roller mill using 2, 3, 4 sets of rolls in a fine grind configuration, or 4 sets of rolls in a coarse grind configuration. The same roller mill was used for all configurations with the appropriate lower rolls completely open when using the 2 or 3 roll pair configurations. Across all studies, mean particle size averaged approximately 540, 435, 270, and 385 μm for the 4 roller mill configurations, respectively. In Exp. 1, 320 pigs (DNA 400 × 200, initially 10.7 ± 0.27 kg BW) were randomly allotted to treatments with 5 pigs per pen and 16 pens per treatment in a 21-d growth trial. While there were no evidence of differences observed for ADG or ADFI, pigs fed corn ground using the 4-high coarse configuration had a marginally significant ($P = 0.091$) improvement in G:F compared to those fed with the 2-high configuration, with others intermediate. In Exp. 2, 90 pigs (PIC 327 × 1050, initially 12.1 ± 0.25 kg BW) were randomly allotted to 1 of 3 diet comparisons to determine feed preference between the 2-high, 4-high fine, and 4-high coarse configurations. When given a choice, pigs consumed more ($P < 0.05$) of the diet containing corn ground through

[1] This work has been published in *Journal of Animal Science*: J. T. Gebhardt, C. B. Paulk, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. C. Woodworth, J. A. DeJong, K. F. Coble, C. R. Stark, C. K. Jones, and S. S. Dritz. 2018. Effect of roller mill configuration on growth performance of nursery and finishing pigs and milling characteristics. J. Anim. Sci. 96:2278-2292. doi:10.1093/jas/sky147.

the 2-high roller mill (67%) or 4-high coarse configuration (63%) compared to corn ground through the 4-high fine configuration. In Exp. 3, 922 finishing pigs (PIC TR4 × [FAST Large white × PIC Line 2], initially 40.1 ± 0.36 kg BW) were used in a 97-d experiment with pens of pigs randomly allotted by initial BW to the same experimental treatments used in Exp. 1. There were 21 pigs per pen and 11 pens per treatment. Pigs fed corn ground with the 2-high configuration had greater ($P < 0.05$) ADG compared to those fed corn ground using the 3-high configuration. Pigs fed corn ground with the 4-high fine configuration had the poorest ($P < 0.05$) ADG. No differences were observed in G:F. Grinding rate (tonne/h) was greatest ($P < 0.05$) for the 4-high coarse configuration, while net electricity consumption (kWh/tonne) was lowest ($P < 0.05$) for the 2-high configuration and greatest for the 4-high fine configuration. In summary, nursery pig G:F tended to be greatest using the 4-high coarse configuration, and finishing pig ADG was maximized using the 2 and 4-high coarse configurations.

Keywords: feed preference, finishing pigs, grinding cost, nursery pigs, particle size, roller mill

INTRODUCTION

It is generally thought that as grain is ground to a fine mean particle size, a linear improvement in nutrient utilization and pig performance will be observed (Rojas and Stein, 2016). Research has demonstrated this benefit when particle size is reduced from 1,000 μm to approximately 400 μm in finishing pigs (Wondra et al., 1995b) and is thought to increase ME from increased starch digestibility as a result of greater surface area for enzymatic digestion (Rojas and Stein, 2016). Generally, as grains are ground to a very fine mean particle size, there becomes an increasing amount of extremely fine particles (Ganesan et al., 2008) which may affect diet palatability and flowability. Hammermills and roller mills are the primary methods used to reduce particle size due to the ability to handle a wide variety of ingredients and

capability to grind to a very small particle size (Heiman and Champion, 2005); however, with a hammermill, the variation in particle size of the final ground grain can be high. By comparison, the roller mill is able to grind grain to a more consistent particle size, and generally has a lower operating cost (Wondra et al, 1995a). Previously, roller mill technology could not achieve mean grain particle size below 600 μm on a consistent basis. Previous research often used a roller mill to grind coarse particle sizes due to the ability to achieve a precise target followed by grinding with a hammermill to grind to a finer particle size (Nemechek, 2016). However, recent introduction of roller mills with 3- or 4-sets of grinding rolls allow for grinding to fine particle size, while minimizing the amount of very fine particles compared to hammermill ground grain. Therefore, the objective of these experiments was to compare various roller mill configurations used to grind corn on milling characteristics, nursery pig feed preference and growth performance, and commercial finishing pig growth performance.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. The roller mill (RMS Roller-Grinder Quadruple Pair, Harrisburg, SD) used to grind corn for all three experiments was located at the New Fashion Pork feed mill located in Estherville, IA and used four pairs of grinding rolls. All corn used in Exp. 1 and 2 was transported to the Kansas State University O.H. Kruse Feed Technology Innovation Center for manufacturing of the complete diets. Diets used in Exp. 3 were manufactured at the New Fashion Pork feed mill (Estherville, IA). Diets for all 3 Exp. were fed in mash form. Experiment 1 was conducted at the Kansas State University Segregated Early Weaning Facility, and Exp. 2 was conducted at the Kansas State University Swine Teaching and

Research Center in Manhattan, KS. Experiment 3 was conducted at New Fashion Pork commercial research facilities located in Round Lake, MN.

In Exp. 1, each pen had tri-bar floors and contained a 4-hole, dry self-feeder and a cup waterer to provide ad libitum access to feed and water. Pens (1.22 × 1.22 m) each contained 5 pigs and allowed approximately 0.298 m^2/pig. In Exp. 2, pens had wire-mesh floors and contained either two, 2-hole, dry self-feeders or two, 4-hole, dry self-feeders balanced among comparisons as well as a nipple waterer to provide ad libitum access to feed and water. Pens (1.52 × 1.22 m) each contained 5 pigs and allowed approximately 0.371 m^2/pig. In Exp. 3, research facilities were double-curtain-sided with completely slatted flooring and deep pits for manure storage. Each pen was equipped with a 5-hole stainless steel dry self-feeder and a cup waterer for ad libitum access to feed and water. Pens (2.44 × 5.59 m) each contained 21 pigs and allowed approximately 0.650 m^2/pig. Daily feed additions to each pen were accomplished and recorded using a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN).

Roller mill specifications

Corn for treatment diets was ground using a roller mill with four sets of grinding rolls. Experimental treatments were diets with the corn fraction ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine grind configuration, or 4 sets of rolls in a coarse grind configuration. The same roller mill was used for all configurations with the appropriate lower rolls completely open when using the 2 or 3 roll pair configurations. The roller mill configurations for each treatment included 2 sets of grinding rolls with roll gaps open to 0.889 and 0.635 mm, listed from top to bottom, 3 sets of grinding rolls with roll gaps open to 0.889, 0.635, and 0.508 mm, 4 sets of grinding rolls with gaps open to 0.889, 0.635, 0.381, and 0.229 mm, and 4 sets of grinding rolls with roll gaps open to 1.02, 0.762, 0.762, and 0.635 mm. All grinding rolls had a 2% left

spiral. The top rolls each had 2.36 corrugations per cm. The second set of grinding rolls had 3.94 corrugations per cm while 1 roll had 4.72 and the 2nd roll had 5.51 corrugations per cm in set 3. The 4th set of rolls had 1 roll with 5.51 and 1 roll with 6.30 corrugations per cm. Thus, the number of corrugations per cm increased from top to bottom. Roll speed differential (approx. 1.5:1) was set to create a shearing action and was 1,126 rpm for the fast roll (40.64 cm diameter sheave) and 763 rpm for the slow roll (59.94 cm diameter sheave). Corn feed rate was set based on a targeted 85% load on the roller mill and amperage for each pair of grinding rolls was collected during grinding. In addition, the roller mill was equipped with sampling ports to allow for collection of ground corn below each set of grinding rolls.

Prior to initiation of the experiment, testing of various roller mill configurations was performed to determine experimental settings. Experimental configurations were established such that corn was ground as consistently as possible using 2 sets of grinding rolls, 3 sets of grinding rolls, 4 sets of grinding rolls, and a final configuration (4-high coarse) which was targeted to the particle size achieved using 3 sets of grinding rolls. This configuration was established to determine if increased throughput could be achieved with an additional set of grinding rolls.

Roller Mill Data and Ground Grain Sample Collection

In addition to grinding corn used in experimental diets, batches of corn were ground on 3 separate occasions using the roller mill for approximately 3 hours using each configuration and routed to a single bin within the feed mill. The corn was then used in production system diets and the process was then repeated for the other configurations. This produced an accurate estimate of throughput over a longer duration of time. Grinding of corn used in treatment diets occurred on 21 dates (1 date for Exp. 1 and 2, 20 dates for Exp. 3). Throughput and electricity consumption

data were collected on the roller mill on 23 separate occasions (20 dates for Exp. 3 and 3 capacity tests) and analysis of samples fed during the growth performance portion of the experiment did not include the samples from the capacity tests. Of the 21 dates when experimental diets were manufactured, corn samples were collected and analyzed on 20 of those dates. Analysis of corn ground for Exp. 1 and 2 was conducted independently of corn ground for Exp. 3. Physical analysis of corn which was fed during Exp. 3 included 19 grinding dates, and the only sample that was collected was from the collection port below the last grinding roll on 15 of those days. On the 4 remaining days, a sample was collected from a port beneath each set of grinding rolls for a detailed analysis of physical characteristics following each set of grinding rolls for each of the experimental configurations. In addition, corn samples were collected between every grinding roll for the 3 capacity tests, for a total of 7 sets of samples collected between each grinding roll. Full physical characteristic analysis was conducted on all 7 sets of samples collected following each grinding roll with the exception of particle size analysis and critical orifice diameter which was unable to be performed for one set of samples due to development of mold within the stored samples.

Electricity consumption was collected on all grinding dates with the exception of nursery corn grinding, for a total of 23 collection dates. Gross roller mill electricity consumption was documented from the automation system output as kW consumption recorded in 1 minute intervals over the full duration of grinding events. Net electricity was calculated by subtracting the power required to operate the roller mill under no load (46 kW) from the gross consumption when grinding each treatment on each grinding date. Net electricity consumption per tonne (kWh/tonne) was then calculated by dividing net electricity consumption (kW) by throughput (tonne/hour).

Ground Grain and Diet Physical Analysis

Particle size analysis, bulk density, angle of repose, and critical orifice diameter (COD) were measured on all ground corn samples at the Kansas State University Swine Lab. In addition, bulk density, angle of repose, and COD were determined for complete diets. Bulk density was determined for ground corn and complete diet using procedures previously described by Clementson et al. (2010) using mass of material contained within a pint cup (Seedburo Model 8800, Seedburo Equipment, Chicago, IL), converted to g/L. Briefly, the sample was poured into a funnel resting above the bulk density cup at which point a slide gate was opened allowing the grain to freely fall into the cup until it overflowed around the circumference. The funnel was then removed and a wood leveling stick was used to remove excess sample and level the sample with the top of the cup with a standardized motion. The weight of the sample in the cup was then recorded.

Particle size was determined using a 13 sieve stack with US sieve numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and pan. A Ro-Tap shaker (W.S. Tyler, Mentor, OH) was used to sift the 100 g samples for 15 min using sieve agitators including bristle sieve cleaners and rubber balls on select sieves. Particle size analysis was conducted with the addition of 0.5 g flow agent (Amorphous silica powder; Gilson Company Inc., Lewis Center, OH) per 100 g sample. Geometric mean particle size by mass (d_{gw}), the geometric standard deviation of particle diameter by mass (S_{gw}), and grain surface area (A_{st}) were calculated using the quantity of sample remaining on each screen following the shaking procedure (ASABE, 2008). Angle of repose was measured by allowing feed or grain to flow freely over a flat circular platform of a known diameter, from which the height of the resulting material was measured and diameter of the pedestal were used to calculate the angle of repose (Appel, 1994).

Critical orifice diameter was measured using a Flowdex device (Hanson Research, Chatsworth, CA) following procedures previously described by Kalivoda, et al. (2015). Briefly, 50 g of sample was measured and allowed to flow through a stainless steel funnel into a cylinder to ensure consistent flow into the cylinder between samples. The sample rested for 30 s in the cylinder, and then the bottom of the cup was opened and the sample was evaluated based on its ability to flow through an opening in a horizontal disc. The discs were 6 cm in diameter and the interior hole ranged from 4 to 34 mm. A negative result was recorded when the sample did not flow through the opening in the disc or formed a cylindrical hole with only the center material falling through the opening. A positive result was recorded when the material flowed through the disc opening forming an inverted cone shape. The procedure began with a small opening and progressively larger discs were used until a positive result was observed. If a positive result was observed, the procedure was repeated using the same disc opening size, until three positive results were consecutively observed and was recorded as the critical orifice diameter.

Animals and Diets

In Exp. 1, pens of pigs [DNA (Columbus, NE) 400 × 200, n = 320, initially 10.7 ± 0.27 kg BW] were randomly allotted to 1 of 4 dietary treatments and fed for 21-d. There were 16 pens per treatment and 5 pigs per pen. The 4 dietary treatments used the identical corn-soybean meal-based formulation that was manufactured from the same batch of ingredients (Table 1). Experimental diets included feed with corn ground using 2 sets of rolls, feed with corn ground using 3 sets of rolls, feed with corn ground using 4 sets of rolls in a fine grind configuration, and with the corn ground using 4 sets of rolls in a coarse grind configuration. Pig weights and feed disappearance were measured on d 0, 7, 14, and 21 to determine ADG, ADFI, and G:F.

As a follow-up to Exp. 1, 90 pigs [PIC (Hendersonville, TN) 327 × 1050, n = 90, initially 12.1 ± 0.25 kg BW] were used in a feed preference study. Pens were randomly allotted to 1 of 3 diet comparisons with 6 pens per comparison and 5 pigs per pen. Each pen contained either two, 2-hole, dry self-feeders or two, 4-hole, dry self-feeders balanced among comparisons with each containing a 1 of the 3 treatment diets to determine feed preference over the 7-d experiment. Experimental diets included the 2-high, 4-high fine, and 4-high coarse manufactured diets with identical formulations to Exp. 1 and used a common batch of ground corn. Diet comparisons tested included the 2-high vs. 4-high fine, 2-high vs. 4-high coarse, and 4-high fine vs. 4-high coarse. Feeders were rotated daily within each pen to reduce any effect of feeder location within pen on intake. Feeders were weighed on d 0, 2, 4, and 7 to determine ADFI of each diet consumed and percentage of total ADFI consumed of each diet, and pig weights were collected on d 0 and 7 of the trial to determine ADG and G:F.

In Exp. 3, a total of 922 pigs [PIC (Hendersonville, TN) TR4 × (FAST {Saskatoon, SK} Large white × PIC Landrace), initially 40.1 ± 0.36 kg BW] were used in a 97-d experiment. Pens were randomly allotted to 1 of 4 experimental treatments by initial BW with 11 pens per treatment and 21 pigs per pen. All diets were the same corn-soybean meal-based diet containing 20% distiller's dried grains with solubles (DDGS). Experimental treatments included the corn fraction of the diets ground using identical roller mill configurations as Exp. 1 and 2. Diets were fed in a 5-phase feeding program, and fed from 32 to 45, 45 to 64, 64 to 82, 82 to 105, and 105 to 127 kg (Table 2). Pigs were weighed and feed disappearance was measured approximately every 2 wk to calculate ADG, ADFI, and G:F. For Exp. 1 and 3, caloric efficiencies (ME- and NE-basis) were calculated by multiplying total feed intake × energy content of the diet (kcal/kg) and dividing by total gain. On d 83 of the trial, pens were weighed and the 6 heaviest pigs from each

pen were removed and transported 550 km to Triumph Foods (St. Joseph, MO) for harvest and collection of carcass data. On d-97, the remaining pigs were transported to Triumph Foods for harvest. Carcass yield was calculated using live weight at the farm and HCW at the plant. At the plant, backfat and loin depth were measured, while percentage lean was calculated using a proprietary formula using HCW, backfat depth, and loin depth.

Chemical Analysis

For all 3 experiments, complete diet samples were collected from multiple feeders within treatment, combined within phase when applicable, and subsampled for analysis. All feed samples were analyzed (Ward Laboratories; Kearney, NE) for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), ash (AOAC 942.05, 2006), Ca (AOAC 965.14/985.01, 2006), P (AOAC 965.17/985.01, 2006), starch (AOAC 996.11, 2006), and ADF (Van Soest et al., 1991). Additionally, diet samples from Exp. 1 and 2 were analyzed for crude fiber (AOAC 978.10, 2006), from which nitrogen-free extract (NFE = 100 – CP – Ash – ether extract - CF) was calculated.

Statistical Analysis

Data were analyzed as a completely randomized design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit in Exp. 1. In Exp. 2, feeder within pen was the experimental unit and pen was included in the model as a random effect. The LSMEANS procedure of SAS was used to evaluate pen means (Exp. 1) and within pen mean difference in ADFI, which was expressed as a percentage of the total consumed for each diet (Exp. 2). In Exp. 3, pens of pigs were blocked by initial BW and allotted to treatment. Data were analyzed as a randomized complete block design using PROC GLIMMIX with pen as the

experimental unit. Hot carcass weight was standardized using a covariate for carcass characteristics including percentage lean, loin depth, and backfat depth.

Roller mill electricity consumption, throughput, and analysis of ground corn samples were analyzed using PROC GLIMMIX with roller mill configuration within grinding day as the experimental unit and grinding date included in the statistical model as a random effect. For analysis of ground corn samples collected following each grinding roll, data were analyzed as an incomplete 4 × 4 factorial arrangement with four roller mill configurations and four roll locations, void of one configuration × roll location combination (2-high configuration, sample following third roll) which was not collected. Degrees of freedom were estimated using the Kenward-Rogers approach. Statistical assumptions were assessed using standard diagnostics on Studentized residuals, and statistical models were expanded to account for heterogeneous residual variance when necessary as described by Goncalves et al. (2016). Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Chemical analysis of diets fed in Exp. 1 and 2 (Table 3) and Exp. 3 (Table 4) resulted in no notable differences among treatments.

Milling Characteristics

Corn ground using the 4-high fine configuration resulted in the slowest ($P < 0.001$) production rate (tonne/h), followed by the 3-high configuration, 2-high configuration, and the 4-high coarse having the greatest production rate (Table 4). Corn ground using the 2-high configuration resulted in the lowest ($P < 0.001$) net electrical energy consumption/tonne of ground corn, followed by the 3-high configuration, 4-high coarse configuration, and the 4-high

fine configuration resulted in the greatest grinding net electricity consumption/tonne ground corn. Within the range of particle sizes generated using the four roller mill configurations in the current study, each 100 µm reduction in particle size increased net electricity consumption/tonne by approximately 0.35 kWh/tonne.

Physical Analysis – Ground Corn and Diets

Corn used in Exp. 1 and 2 ground using the 3-high configuration and the 4-high coarse configuration had similar mean particle size (394 and 403 µm, respectively) and standard deviation (2.73 vs. 2.81, respectively; Table 5). The 4-high fine configuration produced the finest particle size corn, as expected, and also had the lowest standard deviation (267, 2.57 µm, respectively). As particle size was reduced, surface area, expressed as cm^2/gram, increased. Critical orifice diameter was greatest for the 4-high configurations, whereas the 2-high configuration had an improved critical orifice diameter, and the 3-high configuration had the most desirable (lowest) critical orifice diameter flowability. The 4-high fine configuration had the least desirable ($P < 0.001$) angle of repose flowability score, whereas the 2- and 3-high configurations produced the most desirable angle of repose flowability, with the 4-high coarse configuration intermediate. Corn ground using the 4-high fine and 3-high configuration had the lowest ($P < 0.001$) bulk density, whereas the 2-high and 4-high coarse configurations produced ground corn with the heaviest bulk density.

Physical analysis of ground corn fed in Exp. 3 was similar to Exp. 1 and 2 as expected. Corn ground using the 2-high configuration had the greatest ($P < 0.001$) particle size, followed by a reduction in particle size for the 3-high configuration, further reduction for the 4-high coarse configuration, and the 4-high fine configuration having the finest particle size (561, 473, 371, 285 µm). Corn ground using the 4-high fine configuration had the lowest ($P < 0.001$) standard

deviation compared to all other treatments, and the 2-high configuration ground had the greatest, with the 3-high and 4-high coarse configurations being intermediate. The ground corn surface area was greatest ($P < 0.001$) for the 4-high fine configuration, followed by the 4-high coarse, 3-high, and finally the 2-high configuration produced ground corn with the lowest surface area. The 2-high and 3-high configurations had more desirable ($P < 0.001$) critical orifice diameter flowability scores relative to the 4-high configurations. Corn ground using the 2-high configuration had the most desirable ($P < 0.05$) angle of repose flowability, followed by the 3-high, 4-high coarse, and the 4-high fine configuration which produced the least desirable angle of repose flowability. Corn ground using the 4-high fine had the lowest ($P < 0.05$) bulk density, followed by the 3-high and 4-high coarse configurations, while the 2-high configuration produced ground corn with the greatest bulk density. As grain progresses through additional sets of grinding rolls within configuration, a reduction ($P < 0.05$) of particle size and standard deviation was observed as expected (Table 6) in addition to changes in surface area, critical orifice diameter, angle of repose, and bulk density. When physical characteristics of complete diets were analyzed (Table 7), results closely reflected trends observed in analysis of ground corn as expected.

Growth and Carcass characteristics

In Exp. 1, there was no evidence of a difference ($P > 0.10$) in ADG, ADFI or caloric efficiency among pigs fed any of the different roller mill configurations (Table 8). Pigs fed corn ground with the 4-high coarse configuration had marginally significant increased ($P = 0.091$) G:F compared to those fed corn ground with the 2-high configuration (0.667 vs. 0.645, respectively) with others intermediate. In Exp. 2, pigs consumed a greater portion of their total intake (67%; $P < 0.05$) from the diet with corn ground using the 2-high configuration (525 μm)

compared to the 4-high fine configuration (33%; 267 μm; Table 9). There was no difference ($P > 0.10$) in feed consumption between the pigs fed the diet containing 2-high roller mill ground corn (525 μm) and the diet with corn from the 4-high roller mill in a coarse configuration (403 μm; 50.3 vs 49.7%, respectively). Pigs consumed more (63%; $P < 0.05$) of the diet manufactured using corn ground using the 4-high coarse configuration (403 μm) relative to the 4-high fine grind configuration (267 μm; 37%).

In Exp. 3, from d 0 to 56, pigs fed diets containing corn ground with the 2-high roller mill configuration had greater ($P < 0.05$) ADG compared to pigs fed corn ground using the other configurations (Table 10). Pigs fed diets containing corn ground with the 2-high and 3-high roller mill configuration also had increased ($P < 0.05$) ADFI compared to pigs fed diets containing corn ground using the 4-high fine configuration, with those fed corn ground using the 4-high coarse configuration being intermediate. There were no differences ($P > 0.10$) in G:F among roller mill configurations for the grower period.

From d 56 to 97, pigs fed diets containing corn ground with the 4-high fine configuration had the poorest ADG ($P < 0.05$), with no evidence of a difference among the other configurations. Pigs fed diets containing corn ground with the 2-high configuration had the greatest ($P < 0.05$) ADFI, followed by the 3-high and 4-high coarse configurations, and the 4-high fine configuration resulted in the lowest ($P < 0.05$) ADFI. There was a marginally significant ($P = 0.071$) treatment effect for G:F, with pigs fed diets ground with the 4-high coarse configuration having increased ($P < 0.05$) G:F compared to the 2-high configuration, with the 3 and 4-high fine configurations being intermediate. Overall (d 0 to 97), pigs fed diets containing corn ground with the 2-high and the 4-high coarse configuration had the greatest ADG ($P < 0.05$), followed by the 3-high configuration which did not significantly differ from the 4-high

coarse configuration, and the 4-high fine configuration had the lowest ($P < 0.05$) ADG. Pigs fed diets with corn ground using the 2-high configuration had the greatest ($P < 0.05$) ADFI, followed by the 3 and 4-high coarse configurations, and pigs fed corn ground with the 4-high fine configuration had the lowest ($P < 0.05$) ADFI. There was no evidence of differences ($P > 0.10$) in G:F or caloric efficiency among roller mill configurations. Pigs fed diets containing corn ground with the 2-high configuration had greater ($P < 0.05$) final BW and HCW than pigs fed diets containing corn manufactured with the 4-high fine configuration. There was no evidence of differences ($P > 0.10$) in carcass yield, backfat, loin depth, or percentage lean among roller mill configurations.

DISCUSSION

The use of roller mills to process cereal grains has been used for many years (Heiman and Champion, 2005) and the technology and capabilities of these machines has continued to evolve and improve. One of the unique aspects of using a roller mill originates with the grinding mechanism. Traditional roller mills set the roll speed equally for each roll within a set of grinding rolls which would effectively crimp the grain. However, when the roll speed is offset so one roll is rotating more rapidly than the other, the grooves within the rolls allow for a shearing action which creates a fine, uniform finished product (Heiman and Champion, 2005). Hammermills reduce particle size by the impact of the grain against the moving hammer and forcing the particles through a screen with a specific opening diameter specific to the desired particle size. Due to this mechanism, the variation in the final ground corn particle size is typically greater as evidenced by a wider distribution of mass remaining on each sieve following particle size analysis. Until recently, feed manufacturers wishing to reduce the mean particle size below approximately 600 μm found it necessary to use a hammermill. However, the addition of

third and fourth roll sets have allowed the mean particle size to be decreased more than what was previously possible, producing a very finely ground, consistent product (Heiman and Champion, 2005).

Generally as the mean particle size of grain is reduced, a greater amount of energy is required and production rate decreases (Hancock and Behnke, 2001). This observation has been experimentally demonstrated by Wondra et al. (1995b) when grinding corn with both a roller and hammermill, Healy et al. (1994) when grinding sorghum using a roller mill, and De Jong et al. (2016) to when grinding wheat using a hammermill. In the current study, roller mill configuration had a significant impact on throughput. Increasing the number of grinding rolls allows the grinding action to be spread over multiple rolls, resulting in the ability to reduce particle size while still achieving greater production rates. The 4-high coarse configuration ground corn to a finer particle size than the 3-high and 2-high configurations and had a greater overall production rate. However, when the 4-high configuration was adjusted to the fine grind settings, production rate decreased significantly as the particle size was reduced to 285 μm. Net grinding electricity consumption was lowest for the 2-high configuration as would be expected due to the coarse particle size, and reduced particle size led to a linear increase in net electricity consumption. It is important to note the challenges associated with setting appropriate roll gaps for the 3-high configuration to optimize motor load across the three sets of grinding rolls due to the configuration of drive motors and pulleys. Each motor used in the experimental roller mill powered a grinding roll on two separate roll pairs through the pulley mechanism, therefore the 3-high configuration utilized power from four total motors which is why net electrical consumption is reported. This method provides an accurate assessment of net power consumption for all roller mill configurations used. The lack of independent adjustment capability for each roll pair may

have led to a reduced throughput and increased net electricity cost/tonne for the 3-high configuration. Such challenges would not be present with roller mills designed with only three pairs of grinding rolls. Even given the challenges associated with proper roll adjustment, the current experiment demonstrates both that roller mill configuration impacts production rate and reduced particle size targets require a greater amount of electrical power compared to coarser targets. The impact of roller mill configuration on production rate can be explained by a combination of resulting particle size and number of grinding rolls performing work.

As the number of grinding rolls is increased, the capability of the roller mill to reduce particle size of the ground material increased. Additionally, as the mean particle size is reduced, the standard deviation decreases as a result of a greater proportion of the material residing on the lower sieves shifting the distribution more towards the finest particle sizes and reducing the standard deviation. Thus, the lowest standard deviation is perhaps not an accurate measure or predictor for animal growth performance or material handling characteristics due to the decrease when distribution is shifted towards fine particle sizes without other physical analysis characteristics being considered such as particle size, flowability measures including compressibility, critical orifice diameter, or angle of repose, or bulk density.

An important consideration producers face when manufacturing feed is flowability of the material in the feed mill, feed handling systems, and feeder. Generally, as the particle size is reduced, the flowability of the diet is reduced (Rojas, 2016). Corn ground using 4 sets of grinding rolls (fine and coarse configurations) had the poorest flowability as indicated by critical orifice diameter. The 4-high fine configuration had the poorest angle of repose measurement. Additionally, bulk density was lowest for the 4-high fine configuration and greatest for the 4-high coarse and 2-high configurations, with the 3-high configuration being intermediate. When

corn ground using various roller mill configurations was manufactured into a complete swine diet, a similar trend of reduced flowability and bulk density, as well as increasing angle of repose was observed when grain was ground to a finer particle size using an increasing number of grinding rolls. Therefore, roller mill configuration can have a substantial impact on grain and diet flowability, which is driven by the particle size characteristics of the ground grain. Although there was a substantial impact of roller mill configuration on the flowability of the ground corn and complete diets, there were no issues with flowability within the feed mill or at the production site throughout each of the three current experiments.

When grain ground to decreasing particle size is fed to nursery pigs, a characteristic reduction in intake has been observed in sorghum (Healy et al., 1994) and wheat-based (Macromichalis et al., 2000) diets. More recently, as corn is ground to particle sizes of approximately 325 µm, G:F is not improved (De Jong et al., 2013, De Jong et al., 2014a) and a reduction in ADFI is observed resulting in reduced ADG (De Jong et al., 2013). A reduction in feed intake for nursery pigs when fed diets ground to reduced particle sizes is impacted by texture and palatability of the corn fraction of the diet (Sola-Oriol et al., 2009; De Jong et al., 2014a). Bokelman et al., (2015b) observed that, when given a choice, pigs consumed 80% of their ADFI from a diet manufactured with the corn fraction ground to 700 µm, and 20% for the diet with corn ground to 400 µm. Currently, roller mill configuration had a significant impact on nursery pig feed preference, with a general preference for diets manufactured with corn ground using configurations achieving coarser particle sizes. When diets were offered with similar particle sizes ground using different configurations, no preference differences were observed. Nursery pigs are sensitive to diet palatability, and a predictable reduction in feed intake is commonly observed when feeding particle sizes below approximately 500 µm to nursery pigs.

Gain and efficiency responses to feeding nursery pigs reduced particle size diets are typically inconsistent. Gain has been observed to be reduced when the corn fraction is ground to particle sizes approximately 325 µm, driven by a reduction in ADFI with no impact on G:F (De Jong et al., 2013a; De Jong et al., 2013b). Bokelman et al. (2014) observed no significant impact of particle size on ADG or ADFI; however, an improvement in G:F was observed by reducing particle size to 400 µm from 700 µm. The improvement was not observed when diets were fed in pellet form with greatest G:F being observed from pigs fed diets manufactured with 700 µm corn fed in pelleted form. When fed to nursery pigs, diets containing corn ground to very fine mean particle sizes has an impact on feed intake with little impact on G:F, thus negatively impacting growth performance. Further reduction of particle size below 600 µm does not appear to be advantageous for nursery pig diets, even when grinding with a roller mill.

The primary reason cereal grains are ground is to increase the utilization of nutrients, and reducing the particle size has been shown to increase the utilization of energy (GE and ME) in swine diets (Healy et al., 1994, Wondra et al., 1995a, Wondra et al., 1995b, Rojas and Stein, 2016). When considering finishing pig production, a relatively minor improvement in utilization of feed can result in a large economic benefit. Particle size reduction increases the surface area to volume ratio of the grain (Healy, 1994, Wondra et al., 1995c), thus allowing greater contact between grain particles and digestive enzymes. Reduction of particle size has been shown to decrease ADFI when fed to finishing pigs fed corn-based diets (Wondra et al., 1995, De Jong et al., 2013, Nemechek et al., 2016). In the current study, feed intake was significantly impacted by roller mill configuration. However, when corn was ground to reduced particle sizes using a roller mill, an improvement in G:F was not observed, leading to a reduction in gain relative to the coarser-grinding configurations. Wondra et al., (1995b) observed a linear improvement in G:F as

the mean particle size was reduced, resulting in an 8% improvement as mean particle size was reduced from 1,000 to 400 µm. De Jong et al., (2013) observed improved G:F and caloric efficiency on both a ME and NE basis when the particle size was reduced from 596 to 320 µm. Rojas and Stein (2016) observed a linear improvement in G:F for gilts as particle size was reduced from 865 to 339 µm, with no impact of corn particle size on barrow G:F. However, roller mill configuration had no significant impact on overall finishing pig G:F, nor any impact on caloric efficiency on neither a ME nor NE basis. Acosta Camargo et al. (2015) observed an improvement in ATTD of GE in growing pigs when corn and wheat were ground from 700 µm to 300 µm, and in finishing pigs when the grains were ground from 700 µm to 500 µm. However, in finishing pigs, no further improvement in ATTD of GE was observed below 500 µm. Wondra et al., 1995b observed a linear improvement in apparent digestibility of GE with reduced particle size to 400 µm with a similar impact on G:F. Additionally, Acosta Camargo et al. (2015) observed an improvement in ATTD of GE when using a roller mill to reduce particle size from 700 µm to 300 µm in corn and wheat, whereas no further improvement in ATTD of GE was observed when grinding finer than 500 µm using a hammermill. The ability of roller mills to reduce particle size in a very consistent manner without the quantity of very fine materials relative to a hammermill does not appear to consistently provide digestibility or G:F improvements below 500 µm. In the current study, roller mill configuration had a numeric impact on G:F and caloric efficiency in finishing pigs as would be consistent with previous research; however, the negative impacts of reduced ADFI lead to poorer ADG resulted in no advantage when grinding corn to very fine particle sizes for nursery or finishing pig diets.

In conclusion, roller mill configuration had a significant impact on milling characteristics including throughput and electricity cost, nursery pig feed preference, and finishing pig growth

performance. The addition of a fourth set of grinding rolls may provide some flexibility to feed manufacturers using roller mills to grind grain; however, limitations of fine grinding (< 600 μm) previously observed with hammermills, including increases in milling costs and reduced throughput and preference away from fine-ground diets with only marginal improvements in G:F, also appear to be present when using roller mills.

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Table 1.1. Diet composition and chemical analysis for Exp. 1 and 2 (as-fed basis)[1,2]

Item	Exp. 1 and 2
Ingredient, %	
Corn	63.75
Soybean meal (47.7% CP)	32.85
Monocalcium phosphate (21.5% P)	1.10
Limestone	0.98
Salt	0.35
L-Lys HCl	0.30
DL-Met	0.12
L-Thr	0.12
Vitamin premix[3]	0.25
Trace mineral premix[4]	0.15
Phytase[5]	0.015
Total	100
Calculated analysis[6]	
Standard ileal digestible (SID) amino acids, %	
Lys	1.22
Ile:Lys	63
Leu:Lys	129
Met:Lys	33
Met & Cys:Lys	57
Thr:Lys	63
Trp:Lys	19
Val:Lys	69
Total Lys, %	1.37
ME, kcal/kg	3,324
NE, kcal/kg	2,408
SID Lys:ME, g/Mcal	3.73
SID Lys:NE, g/Mcal	5.07
CP, %	21.4
Ca, %	0.70
P, %	0.64
STTP P, %	0.47
Chemical analysis[7]	
DM, %	89.6
CP, %	20.8
Ca, %	0.77
P, %	0.61

[1] Experiment 1 treatment diets were fed to 320 pigs [DNA (Columbus, NE) 400 × 200, initial BW 10.7 ± 0.27 kg] for 21-d.

[2] Experiment 2 treatment diets were fed to 90 pigs [PIC (Hendersonville, TN) 327 × 1050, initial BW 12.1 ± 0.25 kg] for 7-d.

[3] Premix provided per kg of premix: 4,409,249 IU vitamin A; 551,156 IU vitamin D3; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 19,842 mg niacin; 11,023 mg pantothenic acid; and 3,307 mg riboflavin.

[4] Premix provided per kg of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 26.4 g Mn from manganese oxide; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[5] HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), provided an estimated release of 0.09% STTD P.

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

[7] A composite sample collected directly from multiple feeders per treatment within experiment, subsampled, and submitted to Ward Laboratories (Kearney, NE) for analysis. Analyzed values were then averaged among treatments and experiments (n = 7 values for each reported value).

Table 1.2. Diet composition, Exp. 3 (as-fed basis)[1]

	BW range, kg				
Item:	32 to 45	45 to 64	64 to 82	82 to 105	105 to 127
Ingredient, %					
Corn	57.81	62.02	65.68	69.05	70.99
Soybean meal (47.5% CP)	19.41	15.52	12.08	8.66	6.91
DDGS[2]	20.00	20.00	20.00	20.00	20.00
Dicalcium phosphate (18.8% P)	0.45	0.25	0.15	- - -	- - -
Limestone	1.20	1.15	1.10	1.30	1.20
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.45	0.43	0.40	0.40	0.35
L-Thr	0.08	0.06	0.04	0.05	0.04
L-Trp	0.03	0.03	0.02	0.03	0.02
MHA dry (Met)	0.08	0.05	0.03	0.02	- - -
Vitamin and mineral premix[3,4]	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100
Calculated analysis[5]					
Standard ileal digestible (SID) amino acids, %					
Lys	1.02	0.91	0.81	0.73	0.65
Ile:Lys	62	62	63	62	65
Leu:Lys	153	163	174	183	200
Met:Lys	31	31	31	31	31
Met & Cys:Lys	56	57	58	60	62
Thr:Lys	62	62	62	63	66
Trp:Lys	18.5	18.5	18.5	18.5	18.5
Val:Lys	69	71	73	73	78
Total Lys, %	1.14	1.02	0.91	0.83	0.74
ME, kcal/kg	3,211	3,223	3,232	3,234	3,239
NE, kcal/kg	2,462	2,491	2,515	2,533	2,546
SID Lys:ME, g/Mcal	3.18	2.82	2.51	2.26	2.01
SID Lys:NE, g/Mcal	4.14	3.65	3.22	2.88	2.55
CP, %	19.0	17.4	15.9	14.6	13.8
Ca, %	0.67	0.58	0.52	0.53	0.49
P, %	0.54	0.49	0.46	0.42	0.42
STTD P, %	0.40	0.36	0.34	0.31	0.31

[1] A total of 922 pigs (PIC (Hendersonville, TN) TR4 × (FAST (Saskatoon, SK) Large white × PIC Landrace), initial BW 40.1 ± 0.36 kg) were used in a 97-d growth experiment in a 5-phase feeding program.

[2] DDGS = distiller's dried grains with solubles.

[3] VTM premix provided an estimated release of 0.11% STTD P.

[4] Premix provided per kg of premix: 4,116,034 IU vitamin A; 588,635 IU vitamin D3; 26,455 IU vitamin E; 1,470 mg vitamin K; 16.2 mg vitamin B12; 17,637 mg niacin; 11,759 mg pantothenic acid; 5,880 mg riboflavin; 83 g Fe from iron sulfate; 60 g Zn from zinc sulfate; 5.0 g Mn from manganese oxide; 108 g Cu from copper sulfate; 200 mg I from calcium iodate; and 200 mg Se from sodium selenite.

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

Table 1.3. Chemical analysis of diets, Exp. 3 (as-fed basis)[1]

	Roller mill configuration[2]			
Item, %:	2-high	3-high	4-high fine	4-high coarse
Phase 1				
DM	88.91	88.98	88.48	88.59
CP	18.7	19.6	18.1	19.0
ADF	5.5	6.4	6.1	5.8
Ca	0.79	0.62	0.60	0.76
P	0.51	0.48	0.49	0.5
Ether extract	3.4	3.4	3.6	3.2
Ash	4.6	4.1	4.2	4.3
Starch	34.1	33.9	35.7	37.2
Phase 2				
DM	87.87	88.19	87.63	87.88
CP	15.6	15.4	16.6	15.7
ADF	5.2	5.4	5.7	5.4
Ca	0.64	0.63	0.70	0.56
P	0.44	0.43	0.44	0.44
Ether extract	3.3	3.4	3.3	3.4
Ash	4.0	3.7	4.0	3.5
Starch	43.1	41.9	41.4	39.7
Phase 3				
DM	88.02	88.01	87.95	88.1
CP	15.9	15.7	16.4	16.6
ADF	5.8	4.7	5.7	6.0
Ca	0.71	0.54	0.56	0.57
P	0.43	0.45	0.44	0.44
Ether extract	3.1	3.1	3.2	3.4
Ash	3.8	3.6	3.6	3.3
Starch	43.9	40.9	39.9	39.9
Phase 4				
DM	88.64	89.54	88.36	88.33
CP	14.9	14.0	13.5	14.5
ADF	3.7	3.1	4.1	3.8
Ca	0.59	0.48	0.51	0.50
P	0.43	0.41	0.41	0.41
Ether extract	4.1	3.4	3.6	3.7
Ash	3.6	3.2	3.3	3.3
Starch	40.4	40.0	41.2	42.5

Phase 5				
DM	89.92	87.65	88.96	88.44
CP	14.3	14.4	13.1	13.4
ADF	3.8	4.2	3.4	3.4
Ca	0.60	0.59	0.58	0.60
P	0.41	0.43	0.40	0.43
Ether extract	3.4	3.8	3.2	3.6
Ash	3.4	3.4	3.3	3.5
Starch	45.1	41.5	45.5	42.0

[1] A composite sample collected directly from multiple feeders per treatment per phase, subsampled, and submitted to Ward Laboratories (Kearney, NE) for analysis.

[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.

Table 1.4. Roller mill electricity consumption, Exp. 1-3.[1]

Item:	Roller mill configuration[2]				SEM	Probability, $P <$
	2 – high	3 – high	4 – high fine	4 – high coarse		
Grinding rate, tonne/h	12.09[b]	10.89[c]	7.41[d]	13.06[a]	0.357	0.001
Net electricity consumption, kWh/tonne[3]	1.11[d]	1.49[c]	2.17[a]	1.59[b]	0.033	0.001

[1] Data collection occurred on 23 dates (20 diet manufacture dates, 3 capacity tests).
[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.
[3] Net electricity consumption = Net kW/throughput. Net kW = gross kW – kW required to operate roller mill when empty. Gross and tare kW values determined from automation system output in 1 minute intervals for full duration of each grinding event.
[a,b,c,d] Means within row without common superscript differ ($P < 0.05$).

Table 1.5. Physical analysis of ground corn used in growth trials, Exp. 1 - 3[1]

Item:	Roller mill configuration[2]				SEM	Probability, $P <$
	2 – high	3 – high	4 – high fine	4 – high coarse		
Exp. 1 and 2						
Particle size (d_{gw}), µm [3]	525	394	267	403	- - -	- - -
Standard deviation (S_{gw}), µm	3.14	2.73	2.57	2.81	- - -	- - -
Surface area, cm^2/g[4]	166.7	190.9	264.9	192.6	- - -	- - -
Critical orifice diameter, mm[5]	24	22	26	26	- - -	- - -
Angle of repose, degrees[6]	52.0^{c}	52.8^{c}	57.6^{a}	54.3^{b}	0.41	0.001
Bulk density, g/L[7]	509.2^{a}	488.7^{b}	485.9^{b}	507.6^{a}	1.51	0.001
Exp. 3						
Particle size (d_{gw}), µm	561^{a}	473^{b}	285^{d}	371^{c}	10.7	0.001
Standard deviation (S_{gw}), µm	3.04^{a}	2.96^{b}	2.58^{c}	2.97^{b}	0.037	0.001
Surface area, cm^2/g	151.8^{d}	175.9^{c}	247.9^{a}	224.0^{b}	6.13	0.001
Critical orifice diameter, mm	23.3^{b}	23.4^{b}	25.6^{a}	25.9^{a}	0.49	0.001
Angle of repose, degrees	48.0^{d}	50.5^{c}	54.8^{a}	53.7^{b}	0.33	0.001
Bulk density, g/L	516.4^{a}	508.3^{b}	483.2^{c}	509.3^{b}	4.72	0.001

[1] Analysis included only samples of ground corn which were fed during the growth trials and collected at the bottom of the roller mill (1 grinding date for Exp. 1 and 2 corn, 19 grinding dates for Exp. 3).

[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.

[3] Particle size was determined using a Ro-Tap Shaker (W.S. Tyler, Mentor, OH) with 13 sieves and a pan with a shake time of 15 minutes, using 0.50 grams amorphous silica powder (Gilson Company, Inc., Lewis Center, OH) added as sieving agent to 100.0 gram grain sample.

[4] ASABE (2008) Standard S319.4.

[5] Critical orifice diameter measured using Flowdex (Hanson Research, Chatsworth, CA) and represents smallest diameter disc in which 50 grams of material freely flows on three consecutive attempts.

[6] Samples ran in triplicate, thus n = 3 for each treatment.

[7] Samples ran in quintuplicate, thus n = 5 for each treatment.

[a,b,c,d] Means within row without common superscript differ ($P < 0.05$).

- - - Analysis was only performed on the single sample, therefore SEM and *P*-values were not to be determined.

Table 1.6. Characterization of roller mill ground corn, Exp. 3[1]

Item:	Roller mill configuration[2]				SEM	Probability, $P <$		
	2 – high[3]	3 – high	4 – high fine	4 – high coarse		Treatment	Location	Treatment × Location
Particle size (d_{gw}), μm[4]								
Roll set 1	657[b]	716[a,b]	659[b]	741[a]	25.8	< 0.001	< 0.001	< 0.001
Roll set 2	518[c,d,e]	495[d,e]	501[d,e]	543[c,d]				
Roll set 3	- - -	397[g]	337[h]	425[f,g]				
Roll set 4	574[c]	459[e,f]	295[h]	416[f,g]				
Standard deviation (S_{gw}), μm								
Roll set 1	3.98[b]	3.99[a,b]	4.12[a]	4.05[a,b]	0.084	< 0.001	0.002	< 0.001
Roll set 2	3.15[d,e]	3.27[c,d]	3.30[c]	3.34[c]				
Roll set 3	- - -	3.06[e,f]	3.02[f,g]	3.24[c,d]				
Roll set 4	3.02[f,g]	2.81[h]	2.59[i]	2.90[g,h]				
Surface area, cm^2/g[5]								
Roll set 1	181.9[d,e]	166.2[e,f]	191.3[c,d]	166.0[e,f]	10.70	0.001	< 0.001	< 0.001
Roll set 2	171.9[d,e,f]	187.2[d,e]	187.2[d,e]	175.0[d,e]				
Roll set 3	- - -	215.7[b,c]	249.9[a]	214.4[b,c]				
Roll set 4	148.1[f]	177.2[d,e]	234.7[a,b]	194.1[c,d]				
Critical orifice diameter, mm[6]								
Roll set 1	26.3[a,b]	25.3[a,b,c,d]	26.7[a]	26.3[a,b]	0.857	0.100	0.005	< 0.001
Roll set 2	22.7[f,g,h]	24.0[d,e,f,g]	23.0[e,f,g,h]	24.3[c,d,e,f]				
Roll set 3	- - -	23.7[d,e,f,g]	26.0[a,b,c]	25.0[a,b,c,d]				
Roll set 4	22.3[g,h]	21.3[h]	24.3[c,d,e,f]	24.7[b,c,d,e]				
Angle of repose, degrees								
Roll set 1	47.9[g]	48.3[f,g]	48.4[e,f,g]	48.2[g]	0.63	< 0.001	< 0.001	< 0.001
Roll set 2	48.9[d,e,f,g]	49.9[d,e,f]	49.3[d,e,f,g]	50.2[d]				
Roll set 3	- - -	52.0[c]	54.6[a,b]	53.0[b,c]				

Roll set 4	48.4^{f,g}	50.1^{d,e}	55.1^{a}	53.1^{b,c}				
Bulk density, g/L								
Roll set 1	544.6^{a}	546.5^{a}	545.9^{a}	548.6^{a}	6.49	0.006	< 0.001	< 0.001
Roll set 2	524.9^{b}	517.3^{b,c}	519.1^{b}	522.5^{b}				
Roll set 3	- - -	502.2^{d,e}	500.8^{d,e}	515.0^{b,c}				
Roll set 4	508.3^{c,d}	497.9^{e}	480.7^{f}	504.2^{d,e}				

[1] Analysis included only samples collected on dates of manufacture that collected samples below each grinding roll (7 grinding dates – 7 samples for bulk density and angle of repose, 6 samples for remainder of physical characteristic measurements).

[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.

[3] Samples were not collected following third grinding roll for the 2-high configuration.

[4] Particle size was determined using a Ro-Tap Shaker (W.S. Tyler, Mentor, OH) with 13 sieves and a pan with a shake time of 15 minutes, using 0.50 grams amorphous silica powder (Gilson Company, Inc., Lewis Center, OH) added as sieving agent to 100.0 gram grain sample.

[5] ASABE (2008) Standard S319.4.

[6] Critical orifice diameter measured using Flowdex (Hanson Research, Chatsworth, CA) and represents smallest diameter disc in which 50 grams of material freely flows on three consecutive attempts.

[a,b,c,d,e,f,g,h,i] Means within item without common superscripts differ ($P < 0.05$).

Table 1.7. Physical analysis of diets, Exp. 3[1]

Item:	Roller mill configuration[2]				SEM	Probability, $P <$
	2-high	3-high	4-high fine	4-high coarse		
Exp. 1						
Critical orifice diameter, mm[3]	24	24	26	24	- - -	- - -
Angle of repose, degrees	52.1[d]	54.1[c]	57.1[a]	55.8[b]	0.49	0.001
Bulk density, g/L	603.9[a]	587.0[c]	588.8[c]	598.8[b]	1.28	0.001
Exp. 2						
Critical orifice diameter, mm	24	- - -	28	26	- - -	- - -
Angle of repose, degrees	50.4[b]	- - -	57.0[a]	52.1[b]	0.65	0.001
Bulk density, g/L	617.8[a]	- - -	605.3[c]	609.5[b]	0.74	0.001
Exp. 3						
Overall, phases 1 - 5						
Critical orifice diameter, mm	23.4	23.0	27.0	24.6	2.17	0.114
Angle of repose, degrees	50.6[c]	52.4[b,c]	54.5[a]	52.7[a,b]	0.64	0.001
Bulk density, g/L	541.6[a]	534.4[a,b]	528.0[b]	535.9[a,b]	3.33	0.044
Phase 1						
Critical orifice diameter, mm	20	20	26	24	- - -	- - -
Angle of repose, degrees	48.6[b]	49.3[b]	50.3[a]	50.4[a]	0.31	0.001
Bulk density, g/L	565.6[a]	561.1[b]	557.8[c]	565.3[a]	1.09	0.001
Phase 2						
Critical orifice diameter, mm	26	28	30	26	- - -	- - -
Angle of repose, degrees	53.1[c]	54.6[b]	56.3[a]	52.8[c]	0.47	0.001
Bulk density, g/L	547.5[a]	530.2[d]	534.9[c]	539.7[b]	1.04	0.001
Phase 3						
Critical orifice diameter, mm	28	28	28	26	- - -	- - -
Angle of repose, degrees	52.6[c]	56.3[a]	57.0[a]	53.4[b]	0.27	0.001
Bulk density, g/L	538.5[a]	529.9[c]	528.7[c]	535.6[b]	0.79	0.001
Phase 4						
Critical orifice diameter, mm	22	22	28	26	- - -	- - -
Angle of repose, degrees	46.8[c]	49.5[b]	53.9[a]	53.4[a]	0.32	0.001
Bulk density, g/L	538.5[a]	525.8[b]	514.6[d]	518.2[c]	0.73	0.001
Phase 5						
Critical orifice diameter, mm	28	24	30	28	- - -	- - -

Angle of repose, degrees	51.8[c]	52.0[c]	54.8[a]	53.3[b]	0.25	0.001
Bulk density, g/L	518.2[c]	525.0[a]	504.3[d]	520.7[b]	0.77	0.001

[1] A composite sample collected directly from all feeders per treatment was used for analysis.

[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.

[3] Critical orifice diameter measured using Flowdex (Hanson Research, Chatsworth, CA) and represents smallest diameter disc in which 50 grams of material freely flows on three consecutive attempts.

[a,b,c,d] Means within row without common superscripts differ ($P < 0.05$).

[- - -] Analysis was only performed on the single sample, therefore SEM and *P*-values were not to be determined.

Table 1.8. Effects of roller mill configuration on growth performance in nursery pigs, Exp. 1[1]

	Roller mill configuration[2]					
Item:	2-high	3-high	4-high fine	4-high coarse	SEM	Probability, $P <$
BW, kg						
d 0	10.7	10.7	10.7	10.7	0.27	1.000
d 21	23.3	23.3	23.2	23.3	0.56	0.998
d 0 to 21						
ADG, kg	0.60	0.60	0.58	0.60	0.011	0.474
ADFI, kg	0.93	0.92	0.90	0.90	0.024	0.317
G:F	0.645[b]	0.651[a,b]	0.649[a,b]	0.667[a]	0.0083	0.091
Caloric efficiency[3]						
ME, kcal/kg gain	5,079	5,032	5,046	4,916	64.6	0.108
NE, kcal/kg gain	3,737	3,703	3,713	3,618	47.6	0.108

[1] A total of 320 nursery pigs [DNA (Columbus, NE) 400 × 200, initial BW 10.7 ± 0.27 kg] were used in a 21-d study with 5 pigs per pen and 16 replications per treatment.

[2] Corn used in diets was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, and 4 sets of rolls in a coarse configuration.

[3] Caloric efficiency is expressed as kcal/kg gain, using energy values for ME and NE from NRC (2012) Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

[a,b] Means within row without common superscript differ ($P < 0.05$).

Table 1.9. Effects of roller mill configuration on feed intake preference in nursery pigs, Exp. 2[1,2,3]

Item:	2-high	4-high fine	4-high coarse	SEM	Probability, $P <$
ADFI, kg					
Comparison 1	0.52	0.26	- - -	0.032	0.001
Comparison 2	0.40	- - -	0.39	0.026	0.779
Comparison 3	- - -	0.29	0.50	0.038	0.003
ADFI, %[4]					
Comparison 1	67.0	33.0	- - -	3.88	0.001
Comparison 2	50.3	- - -	49.7	2.95	0.882
Comparison 3	- - -	37.1	62.9	3.91	0.001

[1] A total of 90 pigs [PIC (Hendersonville, TN) 327 × 1050, initial BW 12.1 ± 0.25 kg] were used in a 7-d preference trial with 5 pigs per pen and 6 replications per comparison.

[2] Corn used in diets was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, and 4 sets of rolls in a coarse configuration.

[3] Feeders were rotated once daily within each pen to eliminate any location effects of feeder.

[4] ADFI, % is a percentage of total feed intake for each treatment within a comparison.

Table 1.10. Effects of roller mill configuration on growth performance of finishing pigs, Exp. 3[1]

Item:	Roller mill configuration[2]				SEM	Probability, $P <$
	2 - high	3 - high	4 - high fine	4 - high coarse		
BW, kg						
d 0	40.1	40.1	40.1	40.1	0.36	1.000
d 56	97.3[a]	95.7[b]	95.0[b]	95.6[b]	0.51	0.004
d 97	132.3[a]	130.3[a,b]	128.0[b]	130.4[a,b]	0.93	0.022
d 0 to 56						
ADG, kg	1.01[a]	0.99[b]	0.97[b]	0.99[b]	0.006	0.003
ADFI, kg	2.53[a]	2.49[a]	2.43[b]	2.48[a,b]	0.019	0.007
G:F	0.398	0.396	0.400	0.399	0.0023	0.758
d 56 to 97						
ADG, kg	0.95[a]	0.94[a]	0.89[b]	0.94[a]	0.013	0.005
ADFI, kg	3.24[a]	3.11[b]	2.98[c]	3.11[b]	0.035	0.001
G:F	0.294[b]	0.301[a,b]	0.299[a,b]	0.303[a]	0.0024	0.071
d 0 to 97						
ADG, kg	0.99[a]	0.97[b]	0.94[c]	0.97[a,b]	0.007	0.001
ADFI, kg	2.81[a]	2.73[b]	2.65[c]	2.73[b]	0.021	0.001
G:F	0.351	0.354	0.355	0.356	0.0017	0.152
Caloric efficiency[3]						
ME	9,206	9,135	9,094	9,084	43.3	0.145
NE	7,180	7,125	7,092	7,085	33.8	0.136
Carcass characteristics[4]						
HCW, kg	95.5[a]	94.3[a,b]	92.7[b]	93.9[a,b]	0.68	0.036
Carcass yield, %	72.18	71.89	72.65	72.55	0.497	0.562
Backfat, mm	19.85	20.22	19.72	20.37	0.338	0.367
Loin depth, mm	59.2	58.8	60.3	59.5	0.88	0.559
Lean, %[6]	52.41	52.20	52.64	52.22	0.256	0.472

[1] A total of 922 finisher pigs (PIC (Hendersonville, TN) TR4 × [FAST (Saskatoon, SK) Large white × PIC Landrace], initial BW 40.1 ± 0.36 kg) were used in a study with 21 pigs per pen and 11 replications per treatment.

[2] Corn was ground using 2 sets of rolls, 3 sets of rolls, 4 sets of rolls in a fine-grind configuration, or 4 sets of rolls in a coarse configuration.

[3] Caloric efficiency is expressed as kcal/kg gain, using energy values for ME and NE from NRC (2012) Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

[4] The largest 6 pigs were marketed from each pen on d-83. All remaining pigs were marketed from each pen on d-97. Carcass characteristics other than yield were adjusted by using HCW as a covariate.

[a,b,c] Means within row without common superscript differ ($P < 0.05$).

Chapter 2 - Determining the impact of commercial feed additives as potential porcine epidemic diarrhea virus (PEDV) mitigation strategies as determined by polymerase chain reaction analysis and bioassay[2]

ABSTRACT

Mitigation of porcine epidemic diarrhea virus (PEDV) was assessed using two feed additives (0.5% inclusion of a benzoic acid product, BA; and 0.02% inclusion of an essential oil product, EO; DSM Nutritional Products Inc., Parsippany, NJ), and combination of both products (0.5% BA and 0.02% EO) in spray-dried porcine plasma (SDPP) and a swine gestation diet (FEED) as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bioassay. Viral RNA quantification was performed at seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42) and infectivity was assessed via bioassay with 10-d old pigs. There was a tendency for treatment × feed matrix × day interaction ($P = 0.094$), in which the cycle threshold (Ct) value increased over time in FEED when treated with both feed additives, whereas there was no increase over time observed in SDPP treated with both feed additives. There was a feed matrix × day interaction ($P < 0.001$) in which Ct increased over time in FEED, whereas very little increase over time was observed in SDPP. A tendency for a treatment × feed matrix effect ($P = 0.085$) was observed where FEED treated with the

[2] This work has been published in *Translational Animal Science*: J. T. Gebhardt, J. C. Woodworth, C. K. Jones, M. D. Tokach, P. C. Gauger, R. G. Main, J. Zhang, Q. Chen, J. M. DeRouchey, R. D. Goodband, C. R. Stark, J. R. Bergstrom, J. Bai, and S. S. Dritz. 2018. Determining the impact of commercial feed additives as potential porcine epidemic diarrhea virus mitigation strategies as determined by polymerase chain reaction analysis and bioassay. Transl. Anim. Sci. 3:94-102. doi:10.1093/tas/txy100.

combination of EO and BA had a greater ($P < 0.05$) PEDV Ct value than other FEED treatments, and all SDPP treatments had the lower PEDV Ct values compared to FEED treatments ($P < 0.05$). Overall, the combination of both feed additives was most effective at reducing the quantity of genetic material as detected by qRT-PCR ($P < 0.001$) compared to either additive alone or no feed additive. Virus shedding was observed in the d 7 post-inoculation SDPP treatment that was treated with both feed additives, as well as d 0 untreated FEED and d 0 FEED treated with both feed additives. No other treatment bioassay room had detectible RNA shed and detected in fecal swabs or cecal contents. In summary, the combination of EO and BA enhanced degradation of PEDV RNA in feed but had little impact on RNA degradation in SDPP. Both untreated feed and feed treated with the combination of EO and BA resulted in infection at d 0 post-laboratory inoculation; however, neither set of samples was infective at d 1 post-inoculation. In addition, SDPP harbored greater levels of quantifiable RNA for a longer duration of time compared to FEED, and these viral particles remained viable for a longer duration of time indicating differences in viral stability exist between different feed matrices.

Keywords: feed additive, feed, PEDV, swine

INTRODUCTION

Feed and feed ingredients have been proposed to be contributing factors to the introduction of porcine epidemic diarrhea virus (PEDV) in commercial swine herds (Pasick et al., 2014; Bowman et al., 2015), and this route of infection has been proven possible in experimental settings (Dee et al., 2014; Pillatski et al., 2015; Schumacher et al., 2016). Therefore, potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed manufacturing industries. Research assessing potential mitigation techniques have primarily included two approaches: point-in-time

mitigation strategies and mitigation strategies with a prolonged duration of effect. Point in time mitigation strategies, such as use of thermal processing (Cochrane et al., 2017) or irradiation (Trudeau et al., 2016), may be efficacious at the time treatment is performed, but the diet remains susceptible to re-inoculation post-treatment. Prolonged duration of effect approaches involves the addition of chemical agents to the feed or feed ingredient and remain incorporated through time of consumption such as medium chain fatty acids, essential oils, organic acids, or formaldehyde (Dee et al., 2015; Cochrane et al., 2015; Dee et al., 2016; Cochrane et al., 2016a; Trudeau et al., 2016). With documented evidence of potential for disease transmission via feed or feed ingredients, potential methods to mitigate such risk within a feed manufacturing facility with a commercially available, safe, and efficacious product that has a prolonged mitigation activity would be valuable. Therefore, the objective of this experiment was to determine the impact of a commercial benzoic acid product and an essential oil product as potential chemical mitigation strategies of PEDV in feed and spray-dried porcine plasma as determined by real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bioassay.

MATERIALS AND METHODS

General

Treatment structure was arranged as a 2 × 4 × 7 factorial with two feed matrices (complete diet; FEED and spray dried porcine plasma; SDPP) and chemical treatment factors including no addition of feed additives, addition of a benzoic acid product (BA; 0.5%; DSM Nutritional Products Inc., Parsippany, NJ), addition of an essential oils product (EO, 0.02%; DSM Nutritional Products Inc., Parsippany, NJ), and combination of both products (0.5% BA and 0.02% EO). Due to the laboratory analysis procedures requiring separate experimental units to be analyzed on each day, the final factor in the factorial arrangement was day of analysis (d 0,

1, 3, 7, 14, 21, and 42). Swine diet (Table 1) used in this experiment was manufactured at O.H. Kruse Feedmill located at Kansas State University and verified to be free of PEDV and porcine delta-coronavirus (PDCoV) ribonucleic acid (RNA) as determined via qRT-PCR prior to initiation of the experiment. Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA) was also verified by qRT-PCR to be free of both PEDV and delta-coronavirus RNA prior to use.

Chemical Treatment

A 25.0 g sample of each feed matrix was collected and placed in its appropriate bottle (250 mL Nalgene, square wide-mouth high-density polyethylene; ThermoFisher Scientific, Waltham, MA) and received no virus acting as the negative control. For the feed treatment batches, a benchtop paddle mixer was used as previously described (Schumacher et al., 2016) for mixing feed additives with FEED. Mixing time was 3.0 min, which was previously verified as adequate to achieve a CV of < 10% as described by McCoy (2005), using a chloride mixer efficiency procedure (Quantab; Hach Co., Loveland, CO). A V-mixer (Cross-Flow Blender; Patterson-Kelley Co., East Stroudsburg, PA) was used to mix feed additives with SDPP. A mixer efficiency test was performed using spray-dried bovine plasma and resulted in a uniform mix according to manufacturer's recommendation with a mix time of 7.0 min (MicroTracer-F; Microtracers Inc., San Francisco, CA).

Following mixing of feed matrix and corresponding feed additives, 22.5 g of treated feed matrix was sampled from multiple locations within the mixer and placed in the appropriate bottle to be analyzed on seven sampling days post laboratory inoculation, with 3 replications of each sampling day per feed additive treatment combination. This process was repeated for each feed matrix × feed additive treatment combination. Both the paddle mixer and V-blender were cleaned between treatments initially by high pressure air, then a flush step was performed with

either untreated FEED or SDPP for the paddle mixer and V-blender, respectively, followed by a final cleaning with high pressure air. The mixers were then prepared to mix the subsequent treatment.

Inoculation

Inoculation was carried out at the Kansas State University College of Veterinary Medicine Virology Laboratory. The viral inoculum was cell culture derived USA/IN/2013/19338, passage 8 and had an initial concentration of 10^6 $TCID_{50}$/mL. Fifty mL of concentrated inoculum was mixed with 450 mL of tissue culture media, resulting in a diluted inoculum concentration of 10^5 $TCID_{50}$/mL. Inoculation occurred by pipetting 2.5 mL of diluted viral inoculum into each bottle containing 22.5 g treated feed matrix, resulting in an inoculated feed matrix with a viral concentration of 10^4 $TCID_{50}$/g of feed matrix. Following addition of the viral inoculum to each bottle, the bottles were lightly shaken in a circular pattern for approximately five seconds, after which each bottle was hand shaken and inverted for approximately 10 sec to mix the virus evenly within each bottle.

Real-time PCR analysis

Separate bottles were analyzed on d 0, 1, 3, 7, 14, 21, and 42 post-laboratory inoculation. On each day of analysis, 100 mL phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) was added to each bottle predetermined for analysis on that day. Bottles were shaken for approximately 10 s, at which point they were allowed to settle overnight at 4ºC. The following day, supernatant was pulled and aliquoted for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at -20ºC until the conclusion of the trial, at which point qRT-PCR analysis was performed on one aliquot per sample bottle and the

remaining three samples per bottle were stored at -80ºC until transported to Iowa State University for the initiation of the bioassay portion of the experiment.

After collection of d 42 post-laboratory inoculation aliquots, qRT-PCR was conducted on designated preserved aliquots at Kansas State University Veterinary Diagnostic Laboratory Molecular Diagnostics Lab. Fifty microliters (µL) of supernatant from each sample was loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburg, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions with one modification, reducing the final elution volume to 60 µL. One negative extraction control consisting of all reagents except the sample was included in each extraction. The extracted RNA was frozen at -20°C until assayed by qRT-PCR. Analyzed values represent cycle threshold (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until viral genetic material is detected, thus lower quantities of genetic material are present in the original sample.

Bioassay

A bioassay was performed using selected treatment × time combinations at Iowa State University Veterinary Diagnostic Laboratory to determine the viral infectivity characteristics following protocols previously described (Schumacher et al., 2016; Cochrane et al., 2017). The experimental protocol for the pig bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee.

Seventy-eight crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Also, upon arrival, fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) using a qRT-PCR assay. To further

confirm PEDV negative status, serum was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and TGEV antibody by ELISA conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began.

Briefly, pigs from each experimental treatment were housed in separate rooms with independent ventilation systems. Rooms had solid flooring that was minimally rinsed to reduce risk of PEDV aerosolization. Pigs were fed liquid milk replacer twice daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each pig was administered 10 mL of the PBS supernatant treatment by orogastric gavage using an 8-gauge French catheter 0 d post-bioassay inoculation (dpi). Rectal swabs were collected on d -2, 0, 2, 4, and 6 dpi from all piglets and tested for PEDV RNA via qRT-PCR. Cecal content was collected at necropsy and was evaluated for PEDV genetic material via qRT-PCR.

Statistical Analysis

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine the main effects of feed additive, feed matrix, as well as day post-laboratory inoculation and all associated interactions on PEDV Ct values with individual sample bottle as the experimental unit. Degrees of freedom were approximated using the Kenward-Roger approach. The LSMEANS procedure was used along with the LINES option to separate means which differed significantly given the respective interaction or main effect were significant as determined by an *F*-test. Results for response criteria were considered significant at $P \leq 0.05$ and a tendency from $P > 0.05$ to $P \leq 0.10$.

RESULTS

Quantity of Detectible Viral RNA

There was a tendency for a feed additive × feed matrix × day interaction ($P = 0.094$, Table 2) in which the combination of EO and BA resulted in a reduction of quantifiable RNA on d 21 and 42 at a greater rate in FEED than in the SDPP matrix. There was a significant ($P < 0.001$) feed matrix × day interaction in which the Ct value increased over time in FEED, whereas there was very little increase over time observed in SDPP. A tendency for a treatment × feed matrix effect ($P = 0.085$; Table 3) was observed. When further evaluated using means separation, FEED treated with the combination of EO and BA had a greater ($P < 0.05$) PEDV Ct value than other FEED treatments, and all SDPP treatments had the lower PEDV Ct values compared to FEED treatments ($P < 0.05$). Sufficient evidence of a feed additive × day interaction was not observed ($P = 0.259$). All main effects were highly significant, including feed additive, day, and feed matrix ($P \leq 0.001$, Tables 2 and 3). Overall, the combination of EO and BA was most effective at reducing the quantity of genetic material ($P < 0.001$), regardless of feed matrix or day post-inoculation. Overall, a greater quantity of PEDV RNA was detected in SDPP relative to FEED ($P < 0.001$, Ct = 29.3 ± 0.20 vs. 35.0 ± 0.20, respectively). The main effect of day post-inoculation resulted in an increase in PEDV Ct between d 0, 1, 3, 21, and 42 post-laboratory inoculation ($P < 0.001$; 29.3, 30.7, 31.6, 33.9, and 35.2, respectively). There was no difference in Ct between d 3, 7, and 14 post-laboratory inoculation ($P > 0.05$, 31.6, 32.1, and 32.2, respectively).

Infectivity

Upon completion of PCR testing, sixteen samples were selected for assessment of virus infectivity via a bioassay at Iowa State University. The samples selected were d 0 negative

control (FEED and SDPP which was not inoculated with PEDV), d 0, 1, 3, and 21 FEED samples with no feed additive, d 0, 3, and 21 SDPP samples with no feed additive, d 0, 1, 3, 7, 14, and 21 FEED treated with both EO and BA, and d 7 SDPP samples treated with both EO and BA. Each sample consisted of 3 supernatant aliquots that each were gavaged into a single pig within bioassay room. Positive control samples included untreated FEED and SDPP samples at d 0, 3, and 21 post-laboratory inoculation as well as d 1 FEED positive control for a total of 7 total positive control bioassay rooms. The d 0 and d 1 FEED positive control sample was from the current study, however the other 5 positive control samples were in conjunction with additional research from our group (Cochrane et al., 2017) in which bioassay controls were shared across projects (Ct = 29.4, 34.1, 31.6, 37.3, 37.8; d 0 SDPP, d 3 FEED, d 3 SDPP, d 21 FEED, d 21 SDPP, respectively).

No PEDV RNA was detected in fecal swabs prior to initiation of the bioassay, and negative control pigs remained negative for PEDV genetic material for the full length of the bioassay as assessed by fecal swabs and cecal content collected at necropsy (Table 4). Genetic material was detected in fecal swabs for all three pigs in the d 0 untreated FEED and SDPP bioassay rooms beginning at 2 dpi, and viral shedding was observed for the duration of the bioassay. No d 1 untreated FEED pigs had detectible RNA in fecal swabs or cecal contents throughout the bioassay. All three d 3 post-laboratory inoculation untreated SDPP pigs began shedding virus at 2 dpi, whereas the d 3 post-laboratory inoculation untreated FEED pigs had no detectible RNA in fecal swabs throughout the bioassay or cecal content at necropsy. No d 21 post-laboratory inoculation untreated FEED or SDPP pigs had detectible virus in fecal swabs or cecal contents. Thus, pigs became infected with PEDV with both FEED and SDPP at d 0 post-laboratory inoculation, as well as d 3 post-laboratory inoculation in SDPP.

The d 0 FEED treated with EO and BA pigs (3/3) were shedding PEDV RNA as detected by fecal swabs beginning on 2 dpi and remained infected through necropsy at 7 dpi. Virus shedding was observed 2 dpi in fecal swabs in one pig gavaged with d 7 post-inoculation SDPP sample treated with both EO and BA, and all three pigs were shedding virus at 6 dpi and had virus detectible in cecal contents at necropsy. None of the FEED treated with both EO and BA had detectible RNA in fecal swabs or cecal contents with the exception of d 0 post-laboratory inoculation samples. The combination of EO and BA enhanced degradation of PEDV RNA in swine feed but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of EO and BA resulted in PEDV infection at d 0 post-laboratory inoculation; however, neither set of samples were infective at d 1 post-laboratory inoculation.

DISCUSSION

The very small quantity of virus necessary to cause infection has been determined in cell culture (Thomas et al., 2015) as well as in complete feed (Schumacher et al., 2016). Such documentation provides support for field-based reports of potential infection using feed as a vehicle (Pasick et al., 2014; Bowman et al., 2015) by realization that such minute quantities of foreign material can be incorporated into feed manufacturing facilities through improper biosecurity procedures as previously described (Cochrane et al., 2016b). A significant spatial and spatial-temporal clustering pattern was documented with the initial PEDV epidemic beginning in 2013 (Alvarez et al., 2015), suggesting indirect spread such as aerosols or fomites could be a likely explanation. However, it has been established that pathogens including PEDV and porcine delta coronavirus can be found in feed manufacturing facilities and equipment, including truck pedals and flooring (Greiner et al., 2016). Thus, failure of proper biosecurity by feed delivery

personnel can contaminate a feed manufacturing facility and subsequent deposition of infectious material into swine production facilities may contribute to the spread of the virus. Feed manufacturing and delivery is a complex procedure and a high level of biosecurity in such process is critical to maintenance of high herd health, but establishing such procedures is complex and additional research and education efforts are necessary to fully understand the complexity and methods needed to minimize potential disease transmission events (Dewey et al., 2014). Research evaluating a potential mechanism for introduction of viral pathogens into the United States through a transboundary transportation model using feed, various feed ingredients, and pork products has been described (Dee et al., 2018). Such a model demonstrates the plausibility of viruses to survive the conditions which could be expected based on shipment across large geographic regions and provides important information regarding risk differences among viruses and shipped products such that future mitigation strategies can be tailored to specific virus and product. The use of cost effective, readily implementable, and safe feed additives to minimize risk of disease transmission would be a very useful tool in addition to biosecurity practices.

Although the use of commercial formaldehyde has shown significant efficacy in reducing the quantity of detectible PEDV genetic material (Dee et al., 2015; 2016; Cochrane et al., 2016a), concerns arise when considering the implementation of such procedures including the requirement for specialized equipment within feed manufacturing facilities. Therefore, although effective at mitigating risk of PEDV transmission in an experimental setting, the use of formaldehyde is not a solution for all situations. Other compounds when added to feed and feed ingredients have shown promising efficacy at reducing amount of quantifiable RNA as well as reducing infectivity via swine bioassay – most notably medium chain fatty acids (MCFA;

Cochrane et al. 2015a; Dee et al., 2016; Cochrane et al., 2016a). The downfall with the use of MCFA is there are currently no economical sources that are commercially available.

Additional compounds which have been explored as potential PEDV mitigants in feed and feed ingredients include essential oils (Cochrane et al., 2015). Essential oils are plant-derived compounds that have been reported to possess antimicrobial characteristics against a number of pathogens including bacteria, yeasts, and viruses (Reichling et al., 2009). With specific regard to antiviral capabilities, essential oils have shown efficacy against enveloped viruses – primarily those affecting humans - including herpes simplex virus, dengue virus, Newcastle disease virus, severe acute respiratory syndrome (SARS), SARS-associated coronavirus, and Junin virus by likely inhibiting viral replication (Reichling et al., 2009). With specific regard to PEDV, there is little investigation as to the antiviral properties of various essential oil compounds. Cochrane et al. (2015) evaluated a 2% essential oil blend consisting of equal ratios of garlic oleoresin, turmeric oleoresin, capsicum oleoresin, rosemary extract, and wild oregano essential oils, and observed the greatest reduction of quantifiable genetic material occurred approximately d 14 and beyond in both complete swine diet as well as spray dried blood meal. Little impact was observed in spray-dried animal plasma. In the study herein, the combination of EO and BA had the greatest reduction in quantifiable genetic material late in the study period, similar to observations by Cochrane et al. (2015), suggesting efficacy at reducing quantifiable RNA is not immediate. However, the use of EO alone did not result in a significant reduction in PEDV RNA. Although a dramatic increase in Ct is observed at d 21 and 42 post-laboratory inoculation, it is important to note that the infectivity of the PEDV is lost within complete swine feed by 1 d post-laboratory inoculation and between 7 and 14 d post-laboratory inoculation in SDPP. Thus, although the synergistic effect when combining a BA and EO product is interesting and worthy

of investigation, viability of the virus is reduced beyond the point of infectious capability long before such effect on RNA is observed. Similar to Cochrane et al. (2015), commercial products did not result in a significant increase in cycle threshold in SDPP.

Organic acid feed additives have been long-used for control of pathogens, primarily bacteria including *Salmonella* (Carrique-Mas et al., 2006; Van Immerseel et al., 2006). Synergistic benefits have been observed at controlling *Escherichia coli* O157:H7 when combining organic acids with medium-chain fatty acids (Kim et al., 2013); however, limited documentation of co-administration with essential oils is available, particularly specific to viral pathogens. Cochrane et al. (2015b) used a custom organic acid blend included at 3% including lactic, propionic, formic, and benzoic acids which resulted in greater loss of PEDV genetic material over time compared to control, with the greatest efficacy observed in spray-dried animal plasma compared to other matrices. Trudeau et al. (2016) investigated the use of dietary acidifiers including the commercial products Activate DA (0.4% inclusion; Novus International, St. Charles, MO), KEM-GEST (0.2% inclusion; Kemin Agrifoods, Des Moines, IA), Acid Booster (0.2% inclusion; AgriNutrition, DeForest, WI) and Ultracid P (0.3% inclusion; Nutriaid, Dendermonde, Belgium). Inactivation kinetics were improved with the inclusion of Activate DA, KEM-GEST, and Acid Booster compared to the control samples, indicating that inclusion of dietary acidifiers can increase the rate of inactivation of PEDV when experimentally inoculated in swine feed. In the current study, addition of BA alone did not significantly increase PEDV Ct values in FEED nor SDPP. Inclusion rate of the dietary acidifiers evaluated by Trudeau et al. (2016) ranged from 0.2 to 0.4%, whereas inclusion of BA was 0.5% in the current study. It is unclear if this difference in inclusion rate or other factors such as specific organic acids used or

the specific blend of different organic acids led to the different response than that previously observed by Trudeau et al. (2016).

The survival and inversely degradation and/or loss of quantification ability is dependent upon the feed matrix in which the viral particles are inoculated (Dee et al., 2015; Cochrane et al., 2016a). Dee et al. (2015) observed that soybean meal harbored viable PEDV virus at 180 d post inoculation, whereas complete feed harbored viable virus for 45 d post inoculation. In the current study, the detectible quantity of virus maintains a higher level in SDPP compared to complete swine diet. The exact mechanism by which virus viability is affected by feed matrix is not fully understood. The interaction between viral particles and feed matrix is complex and is worthy of additional investigation. Differences in ingredient composition affects the ability to detect genetic material over time as well as substantially impacts duration of viability at room temperature post-laboratory inoculation.

Research evaluating the possibility of PEDV infection using specialty protein feed ingredients, such as spray-dried plasma of bovine (Pujols and Segales, 2014) and porcine (Gerber et al., 2014; Opriessnig et al., 2014; Foddai et al., 2015) origin, have been investigated. Pujols and Segales (2014) found spray-dried bovine plasma when infected with PEDV (2.8 log10 $TCID_{50}$/mL) was not infective in cell culture at 7 d post inoculation when stored at room temperature, and infectivity was lost within 21 d when held at refrigerated temperatures. In the current study, all complete diet and spray-dried porcine plasma samples were held at room temperature (approx. 23°C) following inoculation until addition of PBS at appropriate day of analysis. Spray-dried porcine plasma not treated with chemical treatment was infective in pig bioassay at 3 d post-laboratory inoculation, whereas infectivity was lost by 21 d indicating infective potential lasted somewhere between 3 and 21 d post laboratory inoculation in untreated

SDPP. Furthermore, SDPP treated with EO and BA was infective at 7 d post-laboratory inoculation, whereas complete swine diet (both untreated and treated with combination of EO and BA) lost infectivity by 1 d post-laboratory inoculation. This direct relationship provides additional evidence that PEDV viability is matrix dependent, and in the current experiment SDPP retained a greater quantity of detectible PEDV genetic material and harbored viable virus for significantly longer than complete swine diet.

In summary, the combination of EO and BA enhanced degradation of PEDV RNA in feed but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of EO and BA resulted in infection at d 0 post-laboratory inoculation; however, neither set of samples were infective at d 1 post-inoculation. Finally, spray-dried porcine plasma harbored a greater level of quantifiable RNA for a longer duration of time compared to complete swine diet, and these viral particles remained viable for a longer duration of time indicating differences in viral stability exist between different feed matrices.

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Table 2.1. Diet composition (as-fed basis)[1]

Item:	Swine gestation diet
Ingredient, %	
Corn	80.40
Soybean meal,46.5% CP	15.60
Monocalcium phosphate, 21% P	1.40
Calcium carbonate	1.15
Salt	0.50
L-Thr	0.03
Trace mineral premix[1]	0.15
Sow add pack[2]	0.50
Vitamin premix[3]	0.25
Phytase[4]	0.02
Essential oil[5]	+/-
Benzoic acid[6]	+/-
Total	100
Calculated analysis, %	
CP	14.1
Crude fiber	2.2
Ether extract	3.0
Ca	0.85
P	0.62
Available P	0.46

[1] Each kilogram contains 26.4 g Mn, 110 g Fe, 110 g Zn, 11g Cu, 198 mg I, and 198 mg Se.

[2] Each kilogram contains 110,000 mg choline, 44 mg biotin, 330 mg folic acid, 990 mg pyridoxine.

[3] Each kilogram contains 4,400,000 IU vitamin A, 660,000 IU vitamin D3, 17,600 IU vitamin E, 1,760 mg menadione, 3,300 mg riboflavin, 11,000 mg pantothenic acid, 19,800 mg niacin, 15.4 mg vitamin B12.

[4] HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

[5] Essential oil product (DSM Nutritional Products, Parsippany, NJ) added to complete diet at 0.02% in appropriate treatments.

[6] Benzoic acid product (DSM Nutritional Products, Parsippany, NJ) added to complete diet at 0.5% in appropriate treatments.

Table 2.2. Effect of benzoic acid and essential oil, feed matrix and day on PEDV detection as determined by qRT-PCR.[1]

	qRT-PCR Ct, day post-inoculation[2]						
Item	0	1	3	7	14	21	42
Matrix × feed additive × day[3]							
FEED[4]							
No feed additive	29.4	32.5	31.9	35.2	35.8	37.2	$39.3^{(1/3)}$
EO	30.0	32.8	33.3	34.1	35.5	37.7	38.3
BA	29.7	31.7	33.5	33.4	35.6	38.0	$40.4^{(1/3)}$
EO + BA	30.2	32.4	33.6	36.0	35.5	$42.6^{(2/3)}$	$45.0^{(3/3)}$
SDPP[5]							
No feed additive	28.7	29.5	29.7	29.1	28.9	28.3	29.4
EO	28.4	29.3	29.3	29.1	28.2	30.3	29.4
BA	28.8	28.6	30.5	28.8	29.0	28.5	30.2
EO + BA	29.1	29.1	31.1	30.7	29.2	28.3	29.7
Matrix × day[6]							
FEED	$29.8^{e,f}$	32.3^{d}	33.1^{d}	34.7^{c}	35.6^{c}	38.9^{b}	40.7^{a}
SDPP	28.8^{f}	$29.1^{e,f}$	30.2^{e}	$29.4^{e,f}$	28.8^{f}	28.9^{f}	$29.7^{e,f}$
Day[7]	29.3^{e}	30.7^{d}	31.6^{c}	32.1^{c}	32.2^{c}	33.9^{b}	35.2^{a}

[1] An initial tissue culture (2.5 mL diluted virus inoculum, 10^5 $TCID_{50}$/mL) was inoculated into 22.5 g of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 0.02% essential oils product (EO), 0.5% benzoic acid product (BA), combination of essential oil and benzoic acid products (EO + BA) (DSM Nutritional products, Parsippany, NJ), or no chemical treatment.

[2] Cycle threshold (Ct) required to detect genetic material. A higher Ct value is indicative of less genetic material present.

[3] Matrix × treatment × day interaction, $n = 3$ for each value. SEM = 0.90, $P = 0.094$.

[4] Swine gestation diet.

[5] Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA).

[6] Matrix × day interaction, $n = 12$ for each value. SEM = 0.45, $P < 0.001$.

[7] Main effect of day, $n = 24$ for each value. SEM = 0.32, $P < 0.001$.

$^{(X/X)}$ Superscripts denote number of samples containing no detectable PEDV genetic material following 45 cycles. A value of 45.0 was assumed for samples with non-detectible RNA for analysis.

^{a,b,c,d,e,f} Means within interaction or main effect lacking a common superscript differ ($P < 0.05$).

Table 2.3. Effect of feed matrix and feed additive combination and effect of feed additive on PEDV detection using qRT-PCR.[1,2]

	Feed additive					
Item	Control	EO	BA	EO + BA	SEM	*P* =
Matrix × feed additive						
FEED[3]	34.5	34.5	34.6	36.5	0.34	0.085
SDPP[4]	29.1	29.1	29.2	29.6		
Treatment	31.8[b]	31.8[b]	31.9[b]	33.0[a]	0.24	< 0.001

[1] An initial tissue culture (2.5 mL diluted virus inoculum, 10^5 $TCID_{50}$/mL) was inoculated into 22.5 g of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 0.02% essential oils product (EO), 0.5% benzoic acid product (BA), combination of essential oil and benzoic acid products (EO + BA) (DSM Nutritional products, Parsippany, NJ), or no chemical treatment. A total of 168 samples were used for the analysis with each treatment represented by a mean of $n = 21$ for the matrix × treatment interaction, and $n = 42$ for the main effect of treatment.

[2] Cycle threshold (Ct) required to detect genetic material. A higher Ct value is indicative of less genetic material present.

[3] Swine gestation diet.

[4] Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA).

[a,b,c] Means within item lacking common superscript differ ($P < 0.05$).

Table 2.4. Effects of benzoic acid and/or essential oil products as potential porcine epidemic diarrhea virus (PEDV) mitigation strategies in swine complete feed and spray-dried porcine plasma as determined by pig fecal swab and cecum content qRT-PCR analysis.[1,2]

	Fecal swabs[3]				Cecum contents
Item	-2 dpi	2 dpi	4 dpi	6 dpi	7 dpi
FEED					
No feed additive					
d 0 no virus	- - -	- - -	- - -	- - -	- - -
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 1	- - -	- - -	- - -	- - -	- - -
d 3	- - -	- - -	- - -	- - -	- - -
d 21	- - -	- - -	- - -	- - -	- - -
EO + BA					
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 1	- - -	- - -	- - -	- - -	- - -
d 3	- - -	- - -	- - -	- - -	- - -
d 7	- - -	- - -	- - -	- - -	- - -
d 14	- - -	- - -	- - -	- - -	- - -
d 21	- - -	- - -	- - -	- - -	- - -
SDPP					
No feed additive					
d 0 no virus	- - -	- - -	- - -	- - -	- - -
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 3	- - -	+ + +	+ + +	+ + +	+ + +
d 21	- - -	- - -	- - -	- - -	- - -
EO + BA					
d 7	- - -	+ - -	+ + -	+ + +	+ + +

[1]An initial tissue culture 2.5 mL diluted virus inoculum (10^5 $TCID_{50}$/mL) was inoculated into 22.5 g of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 0.02% essential oils product (EO), 0.5% benzoic acid product (BA), combination of essential oil and benzoic acid products (EO + BA) (DSM Nutritional products, Parsippany, NJ), or no chemical treatment for a final infectious titer of 10^4 $TCID_{50}$/g. The supernatant from each sample was then collected for pig bioassay on the appropriate day post-laboratory inoculation and preserved until initiation of the bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Pigs were initially 10 d old initial BW = 3.6 kg.

[2] (+) indicates quantifiable RNA was detected in fecal swab or cecal content as determined by qRT-PCR. (–) indicates lack of detection of quantifiable RNA in fecal swab or cecal content. Each symbol (+ or -) indicates an individual pig within bioassay room.

[3] Day post-bioassay inoculation.

Chapter 3 - Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on porcine epidemic diarrhea virus (PEDV) cross contamination during feed manufacturing[3]

ABSTRACT

Various strategies have been proposed to mitigate potential risk of porcine epidemic diarrhea virus (PEDV) transmission via feed and feed ingredients. Wet disinfection has been found to be the most effective decontamination of feed mill surfaces; however, this is not practical on a commercial feed production scale. Another potential mitigation strategy would be using chemically-treated rice hulls flushed through the feed manufacturing equipment. Therefore, the objective of this study was to determine the effects of medium chain fatty acids (MCFA) or formaldehyde-treated rice hull flush batches as potential chemical mitigation strategies for PEDV during feed manufacturing. Feed without evidence of PEDV RNA contamination was inoculated with PEDV. Based on PCR analysis, this feed had a cycle threshold (Ct) = 30.2 and was confirmed infective in bioassay. After manufacturing the PEDV positive feed, untreated rice hulls, formaldehyde treated rice hulls, 2% MCFA (a 1:1:1 blend of hexanoic, octanoic, and decanoic acid) treated rice hulls, or 10% MCFA treated rice hulls were flushed through laboratory scale mixers. For the untreated rice hulls, 3 of 6 samples had

[3] This work has been published in *Journal of Animal Science*: J. T. Gebhardt, R. A. Cochrane, J. C. Woodworth, C. K. Jones, M. C. Niederwerder, M. B. Muckey, C. R. Stark, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. Bai, P. C. Gauger, Q. Chen, J. Zhang, R. G. Main, and S. S. Dritz. 2018. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on porcine epidemic diarrhea virus cross-contamination during feed manufacturing. J. Anim. Sci. 96:4149-4158. doi:10.1093/jas/sky295.

detectable PEDV RNA, while 1 of 6 formaldehyde treated rice hull flush samples and 2 of 6 of the 2% MCFA rice hull flush samples had detectable PEDV RNA. However, PEDV RNA was not detected in any of the 10% MCFA rice hull flush samples. Then, rice hulls treated with 10% MCFA were mixed and discharged through a production scale mixer and bucket elevator following PEDV positive feed. No rice hull flush or feed samples from the mixer following chemically-treated rice hull flush had detectible PEDV RNA. However, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectible PEDV RNA. Dust collected following mixing of PEDV contaminated feed had detectable PEDV RNA (Ct = 29.4) and was infectious. However, dust collected immediately after the 10% MCFA rice hull flush batch had a reduced quantity of PEDV RNA (Ct = 33.7) and did not cause infection. Overall, the use of rice hull flushes effectively reduced the quantity of detectible RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with formaldehyde or 10% MCFA provided additional reduction in detectible RNA. Finally, dust collected after manufacturing PEDV-inoculated feed has the potential to serve as a vector for PEDV transmission.

Keywords: chemical treatment, flush, medium chain fatty acid, PEDV, swine

INTRODUCTION

Feed manufacturing equipment has been shown to be a potential source of porcine epidemic diarrhea virus (PEDV) cross contamination (Schumacher et al., 2017). Wet disinfection has been found to be the most effective feed mill equipment surface decontamination method (Muckey, 2016). However, this is not practical in most current commercial feed production settings. Methods to chemically or thermally mitigate the risk of PEDV transmission in feed and feed ingredients have been investigated, (Cochrane et al., 2015b; 2016a; 2017). These methods

are not universally applicable to all feed manufacturing facilities due to equipment cost or safety concerns. Other research has assessed sequencing batches of PEDV negative feed following an inoculated batch of feed to assess the effectiveness of reducing the risk of viral transmission (Schumacher et al., 2016a). While this may be a practical mitigation technique for feed mills to implement, there remains a significant quantity of viral particles on feed-contact surfaces including dust production and distribution throughout the facility (Schumacher et al, 2017). This dust may pose a risk for contamination of subsequent diets. One potential solution is to use chemical mitigants such as formaldehyde or medium chain fatty acids (MCFA) as a periodic flush step within the feed manufacturing process. Rice hulls were selected as the carrier for this chemical flush because the relatively low cost and high degree of abrasiveness, which may help facilitate the removal of viral contamination on equipment surfaces. Therefore, the objective of this experiment was to determine effects of MCFA- or formaldehyde-treated rice hull flush batches as potential PEDV chemical mitigation strategies during feed manufacturing.

MATERIALS AND METHODS

General

The experiment was conducted at the Kansas State University Feed Safety Research Center (FSRC) in Manhattan, KS. Prior to the experiment, the FSRC was decontaminated following a standard protocol approved by the Kansas State University Institutional Biosafety Committee. Prior to initiation of the experiment, the FSRC was physically cleaned using sweeping and compressed air, then chemically cleaned using a two-step process of a 1:256 dilution of ammonium glutaraldehyde blend (Synergize; Preserve International, Reno, NV) and a 1:32 dilution of sodium hypochlorite solution using procedures outlined by Huss et al, (2017). The facility was then heated to 60ºC for a minimum of 24 h and cooled to room temperature at

which point the environmental surfaces were sampled using swabs (World Bioproducts, Mundelein, IL) moistened with phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) and verified devoid of PEDV viral RNA to ensure efficacy of the disinfection procedures prior to initiation of the experiment. After chemical disinfection, the facility was held in containment mode with negative air pressure and high-efficiency particulate air (HEPA) filters preventing contaminated air from leaving the facility. Containment was maintained throughout the experiment and through the post-decontamination procedures.

The swine diet (Table 1) used in this experiment was manufactured at O.H. Kruse Feedmill located at Kansas State University and was verified to be devoid of PEDV and porcine delta-coronavirus (PDCoV) genetic material as determined via qRT-PCR prior to initiation of the experiment. Rice hulls were also verified to be devoid of detectable PEDV and PDCoV genetic material. The production scale mixer used was a 0.113-m^3 electric paddle mixer (H.C. Davis Sons Manufacturing, model # SS-L1; Bonner Springs, KS) with a mix time of 5 min as previously described (Schumacher et al., 2017). Feed was discharged at a rate of approximately 4.5 kg/min into a bucket elevator (Universal Industries, Cedar Falls, IA) fitted with 74 buckets (114 cm^3 each), and then discharged through a 25.4 cm diameter discharge spout and collected in plastic biohazard bags. Laboratory scale stainless steel paddle mixers (n = 13; Cabela's Inc., Sidney, NE) were validated for mixer efficiency for 2.5 and 5.0 kg batches using a mix time of 5.0 min. Validation of mixers prior to the experiment to achieve a coefficient of variation of less than 10% was done following previously described procedures (McCoy, 2005). The volume of rice hulls and feed added to the mixing systems was designed to reflect the fill volume relative to mixer capacity of paddle mixers in a commercial setting.

Chemical Treatment

The procedures used, while reduced in scale compared to commercial production mills, attempt to replicate commercial conditions as closely as possible. Prior to initiation of the experiment, six 2.5 kg chemically-treated rice hull batches were prepared using 2% MCFA blend (n = 2; 1:1:1 ratio of hexanoic, octanoic, and decanoic acid), 10% MCFA blend (n = 2; same ratio of acids used as in 2% blend), or commercial formaldehyde (n = 2; Sal CURB, Kemin Industries, Inc.; application rate = 3.25 kg/tonne). Untreated rice hulls (2.5 kg; n = 2) were also weighed and prepared prior to initiation of the experiment. Rice hulls (untreated and chemically treated) were stored in double lined bags for 48 h at room temperature (21ºC) until initiation of experiment.

Prior to inoculation with PEDV, batches of feed were, mixed, and discharged through both a laboratory scale mixer and production scale systems. For the laboratory-scale mixers, 500 g of PEDV negative feed was added to each mixer, rotated for approximately 15 s, then disconnected from the drive unit and inverted in a one-step motion to dispose of feed into a waste container. A small quantity of residual feed remained in each mixer after this systematic priming and discharge procedure. Following priming of each laboratory scale mixer, a 2.5 kg batch of PEDV negative feed was added to each mixer and mixed as described above. The mixer then was shut off, drive coupler removed from the drive unit motor, and a subsample was collected from six locations within each mixer for a total sample size of approximately 225 g. The mixer was then fully disconnected and inverted to dispose of feed into a waste container.

After priming and collection of the negative feed sample from laboratory scale mixer, the production scale system was primed, and negative sample collected. A 5 kg batch of PEDV negative feed was added to the production scale mixer, allowed to mix for approximately 15 s,

and subsequently discharged into the bucket elevator and was collected at the discharge spout to prime the mixer and fill the boot of the bucket elevator. A 50 kg batch of PEDV negative feed was then added to the production scale mixer, mixed for 5.0 min, and then discharged into the bucket elevator and collected in bags at the discharge spout. A sample of feed was collected from multiple subsample points within the discharged batch of feed.

Laboratory scale mixer inoculation, flush, and subsequent feed

Porcine epidemic diarrhea virus isolation, propagation, and titration were performed as described elsewhere (Chen et al., 2014). The viral inoculum was cell culture derived (USA/IN/2013/19338, passage 9) and had an initial concentration of 4×10^6 $TCID_{50}$/mL. This isolate has been previously shown to be pathogenic in young pigs (Thomas et al., 2015). A 1:10 dilution was performed using PBS to create 2,500 mL of 10^5 $TCID_{50}$/mL viral inoculum. Inoculation of the feed used similar procedures as those described by Schumacher et al. (2016) and Cochrane et al. (2017a). Briefly in this experiment, inoculation of feed to be used in each of the laboratory scale mixers was performed in 5 kg batches using an additional laboratory scale mixer in which 4.5 kg of PEDV negative feed was added to the mixer and 500 mL of 10^5 $TCID_{50}$/mL diluted viral inoculum was added to create 5 kg of 10^4 $TCID_{50}$/g inoculated feed. This batch was mixed for 5 min, at which point it was split into two samples using a riffle splitter and weighed into 2.5 kg batches, bagged, and stored in a freezer (-12ºC) until inoculated into appropriate laboratory scale mixer. This process was repeated three additional times, to create a total of eight 2.5 kg batches of inoculated feed.

After preparation of laboratory scale mixer inoculated feed, each of 8 laboratory scale mixers was inoculated with feed, flush step performed, and a subsequent batch of feed was mixed and sampled. For each inoculation, a bagged sample of PEDV inoculated feed was

randomly selected from the freezer and placed into the randomly selected laboratory scale mixer. Feed was mixed for 5.0 min, at which point a sample of PEDV-inoculated feed was collected from 6 locations within the mixer. Inoculated feed was then discarded into biohazard waste bags using a complete inversion of the mixer following systematic procedure as described above with no tapping or additional cleaning action. The appropriate flush batch was added to the mixer and mixed for 5.0 min. A sample of the rice hull flush was collected from 6 locations within the mixer as previously described. The remaining flush was then discarded, and a subsequent 2.5 kg batch of PEDV-negative feed was added to the mixer and mixed. After mixing, a sample of the subsequent feed was collected, and remaining feed was discarded. This process was repeated 7 additional times in a random order blocked by repetition number, for a total 8 laboratory-scale mixers with two replicates of each of the four chemical treatments (untreated rice hulls, formaldehyde treated rice hulls, 2% MCFA treated rice hulls, and 10% MCFA treated rice hulls).

Production scale system inoculation, flush, and subsequent feed

For inoculation of the production scale system, a 4.5 kg batch of PEDV-negative feed was added to a clean laboratory scale paddle mixer and 500 mL of 10^6 $TCID_{50}$/mL inoculum was slowly added to create a 5 kg batch of PEDV inoculated feed (10^5 $TCID_{50}$/g). Upon conclusion of the addition of the virus, the batch was mixed for 5.0 min to ensure an even mix of virus into the feed inoculum. The PEDV feed inoculum was then added to 45 kg of PEDV-free swine diet in the production scale mixer to create the 50 kg batch of PEDV positive feed (10^4 $TCID_{50}$/g). The entire batch of PEDV-positive feed was then mixed for 5 min, discharged into the bucket elevator, and collected at the bucket elevator discharge spout in biohazard waste bags. A sample of PEDV-positive feed was collected from multiple locations within the discharged batch of PEDV-positive feed. This sample of PEDV inoculated feed was combined at a 1:1 ratio with

PEDV-inoculated feed (also 10^4 $TCID_{50}$/g) from laboratory scale mixer to create a single PEDV-positive sample. After inoculation of the production scale mixer, 36 kg of ground rice hulls was added directly to the mixer, along with 4 kg of MCFA (1:1:1 ratio of hexanoic, octanoic, and decanoic acid) to create a 10% MCFA rice hull flush with a similar mixer fill volume as a 50 kg batch of feed. After a 5.0 min mix time, 6 samples were collected from various locations within the mixer. The rice hull flush batch was then discharged into the bucket elevator and collected at the bucket elevator discharge spout. Samples of discharged flush material were collected at multiple times during discharge to create a single composite sample. A 50 kg batch of PEDV-negative feed was then added to the production-scale mixer and allowed to mix for 5.0 min. A 225 g sample was collected from the mixer and remaining feed was discharged into the bucket elevator and collected at the bucket elevator discharge spout. Again, a 225 g sample was collected from six locations of the bucket elevator to create a single composite sample. Samples were placed on ice and transported to the laboratory for qRT-PCR analysis preparation. Dust samples were also collected throughout the experiment, including dust collected after mixing of 10^4 $TCID_{50}$/g inoculated feed in both the laboratory and production scale systems, after mixing of 10% MCFA treated rice hulls in the production-scale mixer, and collected from mixing of the subsequent feed following the 10% MCFA rice hull flush. All dust collection surfaces were above the fill level of the mixer; therefore, all collected dust had become airborne before depositing on the collection surfaces. Dust was collected from the same surface after each batch of feed (positive inoculated feed, 10% MCFA rice hull flush, and subsequent PEDV-free feed); therefore, dust collected was produced during the associated mixing process and not from previous manufacturing processes.

Viral RNA quantification

After sample collection, temporary storage on ice, and transport to Kansas State University Molecular Diagnostic Research and Development Laboratory, three 50.0 g subsamples of feed from each collection point was added to individual 500 mL high density polyurethane (HDPE) bottles. Rice hull samples from each collection point were subsampled into three 25.0 g samples and added to individual 250 mL HDPE bottles. After subsampling of all feed and rice hull flush samples into appropriate bottles, varying quantity of PBS (100 or 200 mL for rice hull or feed, respectively) were added to each bottle to create a 20% suspension. Bottles were shaken for approximately 10 s, at which point they were allowed to settle overnight at 4ºC. On the next day, supernatant was collected, and aliquots prepared for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at -20ºC until qRT-PCR analysis was performed within 7 d of inoculation on one aliquot per sample bottle. The remaining three samples per bottle were stored at -80ºC until further use. Dust samples were subsampled into 1 mL aliquots, and 4 mL of PBS was added resulting in a 20% suspension by volume. Samples were processed in a similar manner to feed and rice hull flush bottles, and supernatant pulled the following day to be analyzed via qRT-PCR. The remaining dust was stored in dry form at -80ºC until initiation of the bioassay portion of the experiment. Polymerase chain reaction (PCR) assays were performed at the Kansas State University Molecular Diagnostic Research and Development Laboratory as previously described (Schumacher et al., 2016b, 2017) Reported values represent threshold cycle time (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until viral genetic material was detected, thus representing lower quantities of genetic material in the original sample.

Bioassay

Bioassay procedures used were the same as those previously described (Schumacher et al, 2016b; Cochrane et al., 2017). Bioassay samples were selected after qRT-PCR analysis included a composite positive and negative control from laboratory and production-scale mixers, rice hull flush samples from the untreated, formaldehyde, and 2% MCFA flushes of the laboratory-scale mixers, as well as subsequent feed for all 4 laboratory-scale treatments. (Figure 1). Bioassay samples from the production-scale system included 10% MCFA rice hull flush and subsequent feed both collected from the discharge spout of the bucket elevator. Dust samples included those collected from mixing surfaces after manufacture of 10^4 $TCID_{50}$/g inoculated swine feed, after the 10% MCFA rice hull flush, and subsequent feed after the 10% MCFA rice hull flush. Supernatant samples were allowed to thaw prior to inoculation at room temperature, beginning approximately 3 h prior to inoculation. Dust samples were prepared by combining the three positive control dust samples into a single, homogenous positive control dust sample. A total of three, homogenous, dust samples (positive, 10% MCFA rice hull flush, subsequent feed dust) were then each split into three 5.2 g aliquots, and then adding 20.8 g PBS to create a 1:5 suspension of dust to total mass, with a volume of approximately 25 mL each. A 1 mL sample of the suspension was sampled for qRT-PCR analysis, and the remaining solution was inoculated into the appropriate pig (n = 3 pigs per dust type).

The experimental protocol for the bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee. Forty-two crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no known prior exposure to PEDV. Upon arrival, piglets were ear tagged, weighed and randomly assigned to bioassay treatment rooms. Fecal swabs were negative for

PEDV, porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV) using qRT-PCR analysis. Serum was negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and negative for TGEV antibody by ELISA conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before inoculation. Three pigs were housed per room with all pigs challenged with a single treatment. Each room had an independent ventilation system. Biosecurity protocols were in place to prevent viral spread between rooms. Pigs were fed liquid milk replacer once daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each of 33 pigs (11 rooms) receiving supernatant samples were inoculated on d 0 with 20 mL of the PBS supernatant by orogastric gavage. Each of 9 pigs (3 rooms) which were inoculated with dust samples followed similar procedures; however, the remaining solid fraction of the inoculum was placed in the mouth of each pig and were stimulated to swallow. Rectal swabs were collected daily from all piglets and tested for PEDV RNA via qRT-PCR on d -2, 0, 2, 4, 6, and 7 day post inoculation (dpi). Cecal content was evaluated for presence of PEDV genetic material via qRT-PCR at necropsy on d 7.

Statistical Analysis

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine differences between the treatments. Pairwise comparisons were used to determine differences among flush strategies, with the model protected by the overall *F*-test. A cycle time value of 45 was used in the statistical analysis for samples not containing detectible genetic material. Results for response criteria were considered significant at $P \leq 0.05$.

RESULTS

Viral RNA quantification

After qRT-PCR analysis, the composite negative feed sample did not have detectible RNA, and composite positive control feed sample contained detectible PEDV genetic material (Ct = 30.2, Table 2). Following a PEDV positive batch of feed in laboratory scale mixers, 50% of the untreated rice hull flush samples had detectable PEDV RNA. The amount of detectible genetic material was less ($P < 0.05$) within the untreated rice hull flush sample compared to the PEDV positive batch of feed. One of six formaldehyde treated rice hull flush samples was positive for PEDV genetic material, and two out of six of the 2% MCFA rice hull samples had detectable PEDV RNA. In contrast, none of the 10% MCFA rice hull flush samples had detectible PEDV RNA. Chemically-treated rice hull flushes using formaldehyde and 10% MCFA reduced ($P < 0.05$) the quantity of detectible RNA present in the rice hull flush samples compared to the untreated rice hull flush. However, the 2% MCFA rice hull flush did not reduce ($P = 0.215$) the quantity of genetic material compared to the untreated rice hull flush. Importantly, no feed samples collected after an untreated or chemically treated rice hull flush had detectible PEDV genetic material. After manufacturing a PEDV-positive batch of feed in the production-scale mixer and bucket elevator, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectible RNA. However, none of the rice hull flush samples collected from the mixer or subsequent feed samples from the mixer or bucket elevator discharge spout had detectable PEDV RNA. Dust collected after mixing the positive feed had a large quantity of viral RNA (Table 3). Following the inoculated batch of feed, dust collected immediately following the 10% MCFA rice hull flush batch had a reduced quantity of viral RNA, and subsequent feed following the 10% rice hull flush did not have detectible RNA.

Bioassay

All pigs were free of PEDV genetic material in fecal swabs and PEDV-specific antibodies prior to initiation of the bioassay experiment. On 2 dpi, fecal shedding of PEDV RNA was detected in all 3 positive control pigs. These 3 pigs had detectable PEDV RNA at all subsequent fecal sample collections as well as cecal contents at necropsy. No other flush feed bioassay pigs had detectible RNA in fecal swabs throughout the study or cecal content collected at necropsy. Pigs inoculated with the positive dust collected following mixing of inoculated feed were shedding PEDV by d 2 after oral inoculation and continued to shed through necropsy at 7 dpi in both fecal samples and cecal content. However, pigs inoculated with the dust from the 10% MCFA rice hull flush batches or the subsequent feed batch did not have any PEDV genetic material detected in fecal samples throughout the bioassay or in cecal contents at necropsy.

DISCUSSION

Epidemiologic investigation has indicated feed or feed ingredients associated with PEDV transmission (Bowman et al., 2015; Aubry et al., 2017). Furthermore, transmission through feed and feed ingredients has been demonstrated experimentally (Dee et. al, 2014; Pasick et al., 2014; Pillatzki et al., 2015). Efforts to characterize the minimum infectious dose using bioassay of PEDV in feed have shown a low quantity of PEDV is required to contaminate feed (Schumacher et al., 2016b).

Dee et al. (2014) found samples collected from the interior surface of feed bins has the ability to cause infection in naïve pigs. Previous work from our group has also shown that PEDV genetic material is widely dispersed within a feed manufacturing facility (Schumacher et al, 2017). When batches of feed are inoculated with biological agents, specifically PEDV, a large amount of dust is generated and rapidly fills the manufacturing area depositing on virtually every

surface. We have presumed that this material is widely dispersed through viral particles carried on dust. In support of this presumption the results in this study indicate the presence of PEDV genetic material in dust can be infectious. To our knowledge this is the first data reported that has indicated dust within a feed mill can contain an infectious viral pathogen. Although the current experiment was performed in a controlled setting with equipment that is smaller scale than commercial production facilities, it serves as a proof of concept that should be further evaluated with additional research.

In the current experiment, when dust was collected following manufacturing PEDV inoculated feed, a 10% MCFA-treated rice hull flush, and subsequent batch of feed, the only dust sample which caused infection was dust collected following manufacturing PEDV-inoculated feed. Due to the fact that dust samples were collected from the same location following the appropriate batch, it is reasonable to believe the dust collected at each time point was generated during manufacture of the specific batch alone and did not contain carryover material from previous batches. This demonstrates that dust generated during the 10% MCFA flush and subsequent batch of feed was not infectious. However, in commercial mills dust would accumulate over additional batches and the nature of such dust accumulation was not evaluated during our study.

Current feed manufacturing processes such as grain and ingredient receiving, storage, feed manufacture and final delivery to site of consumption have the potential to incorporate infectious material into the manufacturing system, ultimately leading to potentially infectious feed. Batch to batch feed manufacturing and equipment surface contamination has been demonstrated (Schumacher et al, 2016a; 2017). Also, these surfaces have been shown to be difficult to decontaminate (Huss et al., 2017). In such event that biosecurity measures fail and

pathogens such as PEDV enter a feed manufacturing facility, methods to reduce risk of PEDV transmission by feed or ingredients shift to mitigation strategies. Multiple strategies have been proposed to reduce transmission, typically falling into point-in-time or residual duration of activity strategies. Point-in-time strategies include the use of irradiation and thermal processing, while residual duration of activity strategies is commonly thought of as chemical mitigation in which feed additives such as medium chain fatty acids, formaldehyde, essential oils, and dietary acidifiers are included to reduce risk of disease transmission. Both strategies have advantages and disadvantages, and incorporation into feed safety plans should be specific to a given set of circumstances.

In addition to temporary solutions following introduction of pathogens such as chemical mitigation of feed, the strategy must shift to elimination of the pathogen altogether from the facility. In a similar manner by which pharmaceutical compounds are flushed through feed manufacturing equipment (FDA, 1976), sequencing of feed has been proposed to potentially reduce subsequent cross-contamination of PEDV (Schumacher et al., 2016b). The quantity of detectible genetic material was reduced as subsequent batches of feed were manufactured. Also, genetic material was detectible for a longer duration in samples collected from the discharge spout of the experimental bucket elevator compared to samples collected from the mixer. Samples collected from the mixer caused infection for two subsequent batches of feed, whereas no samples collected from the bucket elevator discharge spout for batches of feed following the inoculated batch caused infection. Thus, it has been shown that contamination within feed manufacturing equipment can be reduced using protocols to minimize contamination in later batches of feed. In relation to the current study, a similar reduction in the amount of quantifiable genetic material was observed with increasing number of batches through the mixing and

handling equipment. Increasing the number of batches through a system reduces the level of contamination within the feed and on feed contact surfaces. However, environmental contamination is still a significant concern. In the current study, the presence of detectible viral RNA in the 10% MCFA treated rice hull flush sample collected from the bucket elevator discharge spout, whereas no genetic material was found when collected from the mixer suggests bucket elevators can be a significant cross contamination source within feed manufacturing systems. The inability of feed manufacturing equipment to be completely cleaned between batches of feed, specifically the boot of bucket elevators is a likely cross contamination source.

To compliment the abrasive characteristics of rice hulls, it was hypothesized that chemical treatment of flush material would provide additional benefit beyond rice hulls alone. The use of chemical treatments to reduce PEDV quantity and infectivity characteristics within feed and feed ingredients has been extensive, including commercial feed additives (Trudeau et al., 2016) as well as MCFA's (Cochrane et al., 2015b; 2017b; Dee et al., 2016) and formaldehyde (Dee et al., 2015; Cochrane et al., 2017b). The use of MCFA's has shown significant potential to provide substantial efficacy in reduction of PEDV transmission and could potentially be implemented in the future. Inclusion rates of MCFA's used in previous PEDV mitigation studies have used a 2% maximum inclusion on a wt:wt basis. In the current study, the 2% inclusion rate reduced the number of samples with detectible RNA compared to rice hulls alone, while no PEDV RNA was detected in the 10% MCFA inclusion. Thus, a gradient in efficacy was observed with greater efficacy as PEDV mitigation flushes with 10% inclusion of MCFA.

The use of commercial formaldehyde products, while efficacious as compounds to reduce disease transmission risk, are not applicable in all situations. Limitations exist such that use of

commercial formaldehyde products are not practical in all feed manufacturing facilities due to the lack of application equipment or low usage in swine or poultry feed making justification of such a system impractical. In the current study, the inclusion rate was based on manufacturer recommendation for complete feed and was found to be more efficacious at reducing the amount in quantifiable genetic material in the flush samples than untreated rice hulls and rice hulls treated with 2% MCFA. This efficacy is consistent with previous literature demonstrating formaldehyde products are efficacious at reducing quantities of detectible genetic material as well as infectivity (Dee et al., 2015; Cochrane et al., 2017b). Although no differences were observed in bioassay among treated and untreated rice hulls, chemical treatment of rice hull flushes including the use of formaldehyde and MCFA's reduced the quantity of detectible genetic material.

In conclusion, the dust collected after manufacturing PEDV-inoculated feed contains a large quantity of viral RNA and has the potential to serve as a vector for PEDV transmission. Also, the use of rice hull flushes effectively reduced the quantity of detectible RNA present after mixing a batch of PEDV-positive feed. Additionally, chemical treatment of rice hulls with formaldehyde and 10% MCFA provided additional reduction in detectible RNA and yielded no infectivity in naïve pigs. Such evidence demonstrates the potential for strategically timed flush steps with material such as chemically treated rice hulls to reduce contamination by pathogens within a feed manufacturing facility, providing a useful decontamination procedure in the event a feed manufacturing facility becomes compromised.

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Table 3.1. Diet composition (as-fed basis)

Item:	Swine gestation diet
Ingredient, %	
Corn	79.40
Soybean meal	15.60
Monocalcium phosphate	1.40
Calcium carbonate	1.15
Choice white grease	1.00
Salt	0.50
L-Thr	0.03
Trace mineral premix[1]	0.15
Sow add pack[2]	0.50
Vitamin premix[3]	0.25
Phytase[4]	0.02
Total	100
Calculated analysis, %[5]	
Crude protein	14.0
Crude fiber	2.2
Ether extract	4.0
Ca	0.85
P	0.62
Available P	0.46

[1] Each kilogram contains 26.4 g Mn, 110 g Fe, 110 g Zn, 11g Cu, 198 mg I, and 198 mg Se.

[2] Each kilogram contains 110,000 mg choline, 44 mg biotin, 330 mg folic acid, 990 mg pyridoxine.

[3] Each kilogram contains 4,400,000 IU vitamin A, 660,000 IU vitamin D3, 17,600 IU vitamin E, 1,760 mg menadione, 3,300 mg riboflavin, 11,000 mg pantothenic acid, 19,800 mg niacin, 15.4 mg vitamin B12.

[4] HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

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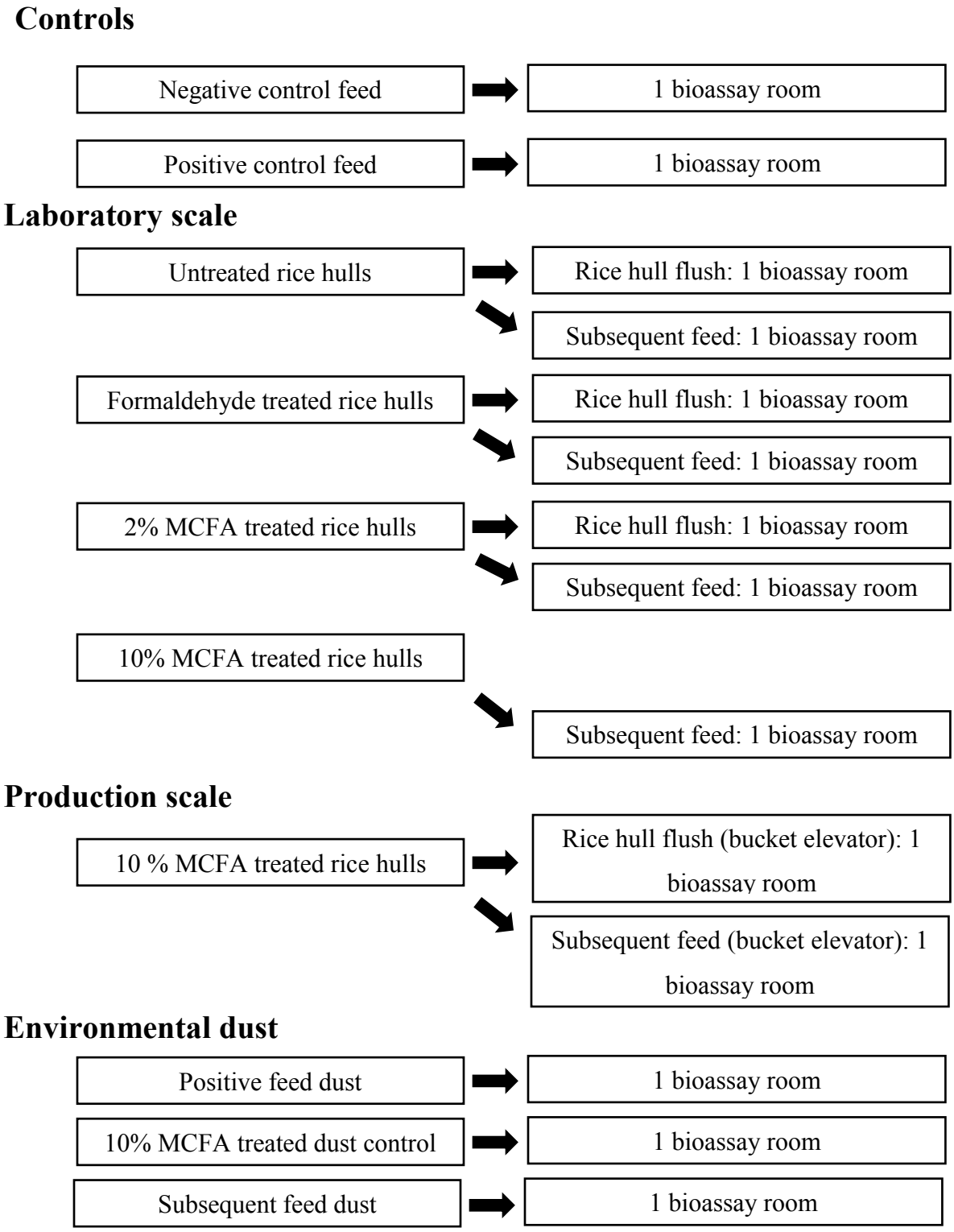

Figure 3-1. Experimental design distinguishing bioassay treatment selection. Laboratory scale mixers and production scale system were used to mix feed inoculated with porcine epidemic diarrhea virus (PEDV), flushed with appropriate rice hull flushes, and mixed a subsequent batch

of feed. Medium chain fatty acid (MCFA) was added on a wt:wt basis. One bioassay room represents a total of 3 pigs.

Table 3.2. Effect of chemically treated rice hull flushes on PEDV RNA detection and infectivity of samples collected in feed manufacturing equipment[1]

	Rice hull treatment			
Item	Untreated	Formaldehyde[2]	2% MCFA[3]	10% MCFA
Prevalence, % positive (positive/total samples)				
Negative feed	0 (0/3)			
Positive feed	100 (3/3)			
Laboratory scale mixer				
Rice hull flush	50 (3/6)	17 (1/6)	33 (2/6)	0 (0/6)
Subsequent feed	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
Production scale mixer				
Rice hull flush	- - -	- - -	- - -	0 (0/3)
Subsequent feed	- - -	- - -	- - -	0 (0/3)
Production scale bucket elevator				
Rice hull flush	- - -	- - -	- - -	33 (1/3)
Subsequent feed	- - -	- - -	- - -	0 (0/3)
Cycle threshold, Ct				
Negative feed	45.0^{a} (-)[4]			
Positive feed	30.2^{d} (+)			
Laboratory scale mixer				
Rice hull flush	41.4^{c} (-)	$43.9^{a,b}$ (-)	$42.4^{b,c}$ (-)	45.0^{a}
Subsequent feed	45.0^{a} (-)	45.0^{a} (-)	45.0^{a} (-)	45.0^{a} (-)
Production scale mixer				
Rice hull flush	- - -	- - -	- - -	45.0^{a}
Subsequent feed	- - -	- - -	- - -	45.0^{a}
Production scale bucket elevator				
Rice hull flush	- - -	- - -	- - -	$42.0^{b,c}$ (-)
Subsequent feed	- - -	- - -	- - -	45.0^{a} (-)

[1] Swine feed was inoculated with porcine epidemic diarrhea virus (PEDV) at a concentration of 10^4 $TCID_{50}$/g and passed through laboratory scale paddle mixers, followed by a rice hull flush, and subsequent batch of PEDV negative swine diet. Batch size was 2.5 kg with a mix time of 5 min.

[2] Sal CURB (Kemin Industries, Inc., Des Moines, IA) was added at recommended level of 3.25 kg/tonne.

[3] Medium chain fatty acid blend (1:1:1 ratio of hexanoic, octanoic, and decanoic acid) added on a wt:wt basis to ground rice hulls.

[4] (+) indicates 3/3 pigs were shedding PEDV genetic material at 2 dpi and continued to shed through 7 dpi and cecal content collected at necropsy contained PEDV genetic material while (-) indicates 0/3 pigs had detectible PEDV genetic material in fecal swabs throughout the full 7 d bioassay as well as did not have detectible PEDV genetic material in cecal contents at necropsy.

[a,b,c] Cycle threshold means lacking common superscript differ ($P < 0.05$). Pooled SEM=0.85.

Table 3.3. Effect PEDV RNA detection and infectivity in environmental dust samples[1,2]

		Fecal swabs				Cecum contents
Item	Inoculum, Ct[3]	-2 dpi	2 dpi	4 dpi	6 dpi	7 dpi
Positive feed dust	29.4	- - -	+ + +	+ + +	+ + +	+ + +
10% MCFA rice hull dust	33.7	- - -	- - -	- - -	- - -	- - -
Subsequent feed dust	45.0	- - -	- - -	- - -	- - -	- - -

[1] Dust samples were collected from the laboratory and production mixers from non-feed contact surfaces.

[2] Infectivity was evaluated in a 10 d old pig bioassay with 3 pigs per dust type. Pigs were individually inoculated on 0 dpi. (+) indicates that an individual pig was found to have detectable PEDV genetic material in the respective sample using qRT-PCR. (-) indicates that an individual pig did not have detectable PEDV genetic material in the respective sample.

[3] Positive feed dust, average of $n = 3$, 10% MCFA rice hull dust, $n = 1$; subsequent feed dust, $n = 1$. PEDV qRT-PCR cycle threshold (Ct).

Chapter 4 - Influence of chromium propionate dose and feeding regimen on growth performance and carcass composition of pigs housed in a commercial environment[4]

ABSTRACT

While chromium feeding study results have been variable, our hypothesis was feeding a regimen that changed dosage over time would result in a larger positive response in growth performance and carcass characteristics. In Exp. 1, 1,206 pigs (PIC 337 × 1050, initially 28.7 kg) were used with 27 pigs per pen and 9 pens per treatment. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in a 5-phase feeding program. Treatments were arranged as a 2 × 2 + 1 factorial with a control diet containing no added chromium propionate (Cr; Kemin Industries Inc., Des Moines, IA), or diets with either 100 or 200 µg/kg added Cr during the grower (dietary phases 1 and 2) and/or finisher (dietary phases 3, 4, and 5) periods. During the grower period, ADG and G:F were similar among pigs fed the control or 100 µg/kg added Cr diets, but decreased in pigs fed 200 µg/kg Cr (quadratic, $P \leq 0.001$). During the finisher period, pigs supplemented with 200 µg/kg added Cr had the greatest ADG and G:F (quadratic, $P \leq 0.019$). Overall, increasing Cr had no effect on ADG or ADFI; but G:F was greatest (quadratic, $P = 0.020$) when pigs were fed 100 µg/kg of added Cr throughout. Carcass characteristics were not influenced by Cr dosage or feeding regimen. In Exp. 2, a total of 1,206 pigs (PIC 359 × 1050, initially 48.9 kg) were used with 27 pigs per pen and 15 pens per treatment. Diets were

[4] This work has been published in *Translational Animal Science*: J. T. Gebhardt, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. A. Loughmiller, A. L. P. de Souza, and S. S. Dritz. 2019. Influence of chromium propionate dose and feeding regimen on growth performance and carcass composition of pigs housed in a commercial environment. Transl. Anim. Sci. 3:385-392. doi:10.1093/tas/txy104.

corn-soybean meal-dried distillers grains with solubles-based and were fed in 4 phases. There were three dietary treatments: a diet with no added Cr for both grower (dietary phase 1 and 2) and finisher (dietary phase 3 and 4) periods, a diet with 200 μg/kg added Cr during the grower and 100 μg/kg added Cr during the finisher periods, or a diet with 200 μg/kg added Cr for both periods. Addition of 200 μg/kg Cr in both periods marginally increased ($P < 0.10$) ADG compared to pigs fed no added Cr. There was no evidence ($P \geq 0.523$) of added Cr influencing overall ADFI and G:F. Percentage carcass yield was reduced ($P = 0.018$) when Cr was added at 200 μg/kg for both periods, with no evidence of differences ($P \geq 0.206$) in other carcass characteristics. In summary, overall G:F was improved in Exp. 1, and ADG in Exp. 2, by added Cr, but there was no evidence that different feeding regimens will consistently result in improved performance. However, these data are consistent with the literature in that added Cr in growing-finishing pigs diets improves, albeit small, ADG or G:F.

Keywords: chromium propionate, duration, finishing pig, level

INTRODUCTION

Chromium (Cr) has been shown to be involved in carbohydrate, lipid, and protein metabolism (Pechova and Pavlata, 2007; NRC, 2012). Historically, the most notable mode of action is its influence on insulin sensitivity as component of the molecule known as glucose tolerance factor (Steele et al., 1977; Hill and Spears, 2001), however additional research has indicated chromodulin is the likely oligopeptide responsible for its activity (Pechova and Pavlata, 2007). With regards to the effects of Cr on swine growth performance, published literature contains significant variability regarding growth and carcass characteristics. Due to the variability in ingredient basal chromium levels and inconsistent performance, there is currently no quantitative estimate for Cr requirements for swine (NRC, 2012). A meta-analysis was

conducted that included 31 different studies evaluating added Cr in finishing pig diets. The meta-analysis suggested variable but overall positive improvements in ADG and G:F, as well as reducing backfat and increasing percentage lean which can be beneficial in some situations with supplemental Cr (Sales and Jancik, 2011). However, Lindemann (2007) indicated that as body mass increases, there are reduced tissue concentrations of Cr. This might suggest that using feeding regimens that combine different Cr dosages and feeding durations could result in even greater improvements in growth or carcass performance. Therefore, the objective of this experiment was to determine the effects of Cr propionate dosage and feeding regimen on growth performance and carcass composition of pigs housed in a commercial environment.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. The studies were conducted at a commercial research-finishing site in southwest Minnesota using two identical barns. The barns were naturally ventilated and double-curtain-sided. Each pen (5.5 × 3.0 m) was equipped with a 4-hole stainless steel feeder and cup waterer for ad libitum access to feed and water and allowed approximately 0.61 m^2/pig. All feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

Animals and Diets

In Exp. 1, a total of 1,206 pigs (PIC, Hendersonville, TN; 337 × 1050) initially 28.7 kg were used in a 125-d growth trial with 27 pigs per pen and 9 pens per treatment. Pigs were split by sex upon arrival at the facility, with 4 blocks of each gender and a final mixed sex gender block. Gender blocks were randomly allotted to groups of 5 pen locations within the barn. Diets

were corn-soybean meal-based and fed in meal form, with dietary phases formulated for 27 to 45, 45 to 61, 61 to 77, 77 to 104, and 104 to 127 kg BW ranges. All diets were formulated to meet or exceed the NRC (2012) nutrient requirement estimates within phase. Ingredient nutrient profiles and standardized ileal digestibility coefficients were derived from NRC (2012), The treatment phases were divided into two specific growth ranges including a grower period (dietary phases 1 and 2) and a finisher period (dietary phases 3, 4, and 5). Treatments were arranged as a 2 × 2 + 1 factorial with main effects of Cr dose (100 or 200 μg/kg of Cr from Cr propionate; Kemin Industries Inc., Des Moines, IA) and feeding regimen (grower or finisher periods) and a control diet containing no added Cr. Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added in phase 5 diets when pigs were an average of 104 kg BW and was fed for the final 38-d of the trial. Diets were manufactured in a commercial feed mill in SW Minnesota (New Horizon Feeds, Pipestone, MN; Table 1).

In Exp. 2, a total of 1,206 pigs (PIC 359 × 1050) initially 48.9 kg were used in an 84-d growth trial with 27 pigs per pen and 15 pens per treatment. Pigs were placed in mixed-gender pens with similar numbers of barrows and gilts in each pen and equalized by treatment. Pens were blocked by average body weight and randomly assigned to treatment at initiation of the experiment. Diets were corn-soybean meal-based and fed in meal form, with dietary phases formulated for 45 to 68, 68 to 91, 91 to 109, and 109 to 127 kg BW ranges. All diets were formulated to meet or exceed the NRC (2012) nutrient requirement estimates within phase. Three dietary treatments were offered which included a control with no added Cr for both grower (dietary phase 1 and 2) and finisher (dietary phase 3 and 4) phases, the control diet plus 200 μg/kg added Cr during the grower and 100 μg/kg added Cr during the finisher periods, or the control diet plus 200 μg/kg added Cr for both the grower and finisher periods. All diets were

manufactured at a commercial feed mill (New Horizon Feeds, Pipestone, MN; Table 2) and were fed in meal form. No ractopamine HCl was used in Exp. 2.

In both experiments, pens of pigs were weighed and feeder measurements were recorded a minimum of every 14-d and such events included dietary phase changes, first marketing, and conclusion of the trial to determine ADG, ADFI, and G:F. The 3 heaviest pigs per pen were selected using visual evaluation by trained personnel and marketed at an average barn weight (Exp 1: 116 kg on d-97; Exp. 2: 110 kg on d-68) following the routine farm protocol with no carcass data collected from these pigs. At the conclusion of the trial (Exp. 1, d-125; Exp. 2, d-84), the remaining pigs were given a tattoo corresponding to pen number and were transported to a commercial packing facility (JBS Swift and Company; Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included HCW, backfat, percentage carcass lean, and loin depth. Backfat and loin depth were measured using an optical probe inserted between the third and fourth ribs from the caudal aspect of the carcass at a distance approximately 7 cm from dorsal midline as described by Coble et al. (2017). Percentage carcass lean was calculated using a proprietary formula using HCW, backfat depth, and loin depth. Additionally, percentage carcass yield was calculated by dividing pen average HCW by pen average live weight collected at the research facilities prior to transport to processing facility.

Chemical Analysis

For both experiments, complete diet samples were collected from multiple feeders within treatment, combined within phase when applicable, and subsampled for analysis. All feed samples were analyzed (Ward Laboratories; Kearney, NE) for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), crude fiber (AOAC 978.10, 2006)

and University of Guelph Agriculture & Food Laboratory (Guelph, ON) for analysis of Cr (US EPA 6020a, 1998).

Statistical Analysis

Data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. In Exp. 1, block was included in the model as a random effect and accounted for gender, location within barn, and initial BW at the time of allotment. Linear and quadratic effects of increasing Cr within growth period were considered using all treatments, as well as linear and quadratic effects of increasing Cr within treatments fed at a constant level for the full duration of the trial. An additional pairwise contrast was analyzed to determine the impact of changing Cr concentrations between the grower and finisher periods. In Exp. 2, weight block was included in the model as a random effect which accounted for initial BW at the time of allotment. Growth performance during the grower period were analyzed to compare 0 vs. 200 µg/kg added Cr. During the finishing period, growth performance characteristics were analyzed using linear and quadratic contrast statements comparing the effect of increasing dietary Cr supplementation (0, 100, and 200 µg/kg Cr). Overall growth performance and carcass characteristics were analyzed using an *F*-test to determine if at least one treatment differed from another, and LSMEANS procedure with the DIFF and LINES options to separate significant differences among treatments (0/0, 200/100, 200/200 µg/kg added Cr, corresponding to grower/finisher Cr, respectively). In both experiments, backfat, loin depth, and percentage lean were adjusted to a common carcass weight for analysis using HCW as a covariate, and percentage yield was calculated by dividing the pen average HCW by pen average live weight as measured at the research barn prior to transport to

processing facility. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Chemical analysis of complete diets revealed no notable differences in proximate analysis including DM, CP, ether extract, and crude fiber among treatments (Tables 3 and 4). Although variable, analyzed Cr values were greater in diets with added Cr, as expected.

Experiment 1

Overall growth performance and carcass characteristics were compared between pigs fed 100/200 and 200/100 μg/kg added Cr during the grower and finisher periods, respectively. No evidence of a difference ($P \geq 0.416$) between treatments was detected, indicating no benefit was observed with changing dosages between growth periods. With no benefit associated with feeding regimen observed, linear and quadratic effects of increasing Cr within growth period were considered using all treatments, as well as linear and quadratic effects of increasing Cr for the full duration using the 3 treatments which had a constant Cr dosage throughout.

Increasing Cr during the grower period resulted in no benefit when 100 μg/kg Cr was fed compared with control fed pigs, but reduced (quadratic, $P < 0.001$; Table 5) ADG and G:F with 200 μg/kg added Cr. No differences ($P \geq 0.229$) in ADFI were detected within the grower period as Cr dosage increased. During the finisher period, pigs fed diets with 100 μg/kg added Cr had the greatest (quadratic, $P < 0.019$) ADG, while G:F was equally improved (quadratic, $P < 0.001$) by either Cr dose. Overall, increasing Cr resulted in no evidence of an effect on ADG or ADFI ($P \geq 0.136$); however, G:F was greatest (quadratic, $P = 0.020$) when pigs were fed 100 μg/kg

added Cr in both grower and finishing phases. There was no evidence of difference ($P \geq 0.115$) in carcass characteristics among different Cr dosages or feeding regimen.

Experiment 2

In Exp. 2, there was no evidence ($P \geq 0.197$) of differences between the treatments for ADG, ADFI, or G:F in the grower period. In the finishing period, addition of Cr resulted in a marginally significant increase (linear; $P = 0.061$) in ADG as Cr increased with no evidence of an effect ($P \geq 0.157$) on ADFI or G:F. For the overall period, addition of 200 μg/kg Cr in both grower and finisher periods increased ($P < 0.05$) ADG compared to pigs fed the control, with pigs fed 200 μg/kg Cr fed in grower followed by 100 μg/kg fed in finisher intermediate. There was no evidence ($P \geq 0.523$) of added Cr on overall ADFI and G:F. Percentage carcass yield was decreased ($P = 0.018$) when Cr was added at 200 μg/kg for both the grower and finishing periods compared to the other treatments. There was no evidence of differences ($P \geq 0.206$) in HCW, loin depth, backfat, or percentage lean among treatments.

DISCUSSION

Chromium is associated with metabolism of glucose, lipids, protein, and nucleic acids (NRC, 2012). The specific role in glucose metabolism historically was believed to be through its presence on the glucose tolerance factor (Steele et al., 1977; Page et al., 1993; Matthews et al., 2001); however, additional research has indicated chromodulin is the likely oligopeptide responsible for activity (Pechova and Pavlata, 2007). With regards to growth performance and carcass characteristics of finishing pigs, recent scientific literature has shown wide variability in efficacy of added Cr. A number of studies have indicated improvements in carcass characteristics as well as growth performance; however, the presence and magnitude of such responses is all but clear. As a result of the variability observed when Cr is added to swine diets, it is thought that the

positive responses might be influenced by dietary nutrient concentrations, environment, and management factors (Lindeman, 2007). Additionally, it is thought that the magnitude of response is related to the length of feeding, dosage, and perhaps even the body weight of the pig (Lindeman, 2007). It is also theorized that some variability in response to added Cr may be due to its particle size which may affect absorption characteristics (Hung et al., 2015).

One of the major challenges with evaluating the effects of added Cr on growth performance may be attributed to the significant variability in the quantity of Cr present in feedstuffs commonly used in swine diets. Traditional corn-soybean meal-based diets can vary in Cr content from 750 to 3,000 μg/kg (NRC, 2012). Although natural sources of dietary Cr can be very variable, it is believed that only a small fraction of the total Cr present naturally is available for utilization (NRC, 2012). Lindemann (2007) proposed that organic forms of Cr are believed to be much more bioavailable. Thus, evaluation of dietary Cr though laboratory evaluation can be quite misleading, and Cr level is routinely described as quantity of organic Cr added as opposed to total analyzed Cr. In the current series of experiments, variability was observed in Cr analysis as measured by mass spectrometry, but in general analyzed Cr concentrations increased as the level of added Cr increased.

Chromium propionate was granted permission by the United States Food and Drug Administration in 2000 to be marketed without objection for inclusion in swine diets at inclusion levels up to 200 μg/kg (Lindemann, 2007), and similar bioavailibity to Cr picolinate has been observed (Matthews et al., 2001). However, evaluation of different sources of Cr provides evidence that when added at very high levels, tissue concentration of Cr differed among the various sources (Lindemann et al., 2008). Additional investigation into added Cr propionate in finishing pig diets has observed variable effects on growth performance and carcass

characteristics (Shelton et al., 2003; Matthews et al., 2005, Jackson et al., 2009). Therefore, because Cr propionate has been shown to be a bioavailable source of Cr in swine, further investigation into the effects of supplementation under commercial conditions was the primary objective of the current series of experiments.

In addition to a large degree of variability in Cr composition of feed ingredients and questionable bioavailability, the historical influence of added Cr on growth outcomes and carcass composition is also quite variable (Lindeman, 2007). A number of peer-reviewed publications show both benefits and no response when adding Cr on both growth performance and carcass characteristics. Greater detail regarding the mixed results of these studies is provided in NRC (2012). In order to summarize the body of published evidence, a meta-analysis on added dietary Cr on carcass characteristics and growth performance of finishing swine was conducted by Sales and Jancik (2011). Their evaluation included studies which added chromium in the form of Cr-methionine chelate, Cr-nanocomposite, Cr-nicotinate, Cr-propionate, Cr-tripicolinate, and Cr-yeast. Cumulative findings of the 31 studies analyzed observed a reduction in backfat thickness, and an increase in percentage carcass lean and loin muscle area with added Cr. In the series of experiments herein, the only carcass characteristic that was influenced by added Cr was a reduction in percentage carcass yield and only in Exp. 2. In the review by Sales and Jancik (2011), they observed that the later in the finishing period when Cr supplementation was initiated, the greater the magnitude of decreased fat and increased carcass lean. Boleman et al. (1995) found that supplementation of 200 μg/kg Cr-picolinate only in the finisher period resulted in greater carcass percentage muscle, lower tenth rib backfat, and lower total carcass fat percentage compared to both control and pigs fed 200 μg/kg added Cr-picolinate in both the grower and finisher periods.

In conclusion, growth performance was moderately influenced with the addition of Cr-propionate in swine diets. Carcass composition was largely unaffected by added Cr with the exception of reducing percentage carcass yield in Exp. 2. The specific dosage in which ADG and G:F was maximized varied from 100 μg/kg in Exp. 1 to 200 μg/kg added Cr in Exp. 2. The results of these trials do not provide evidence that different feeding regimens will consistently result in improved performance. Under commercial swine production conditions in the current series of experiments, addition of Cr-propionate in finishing pig diets has the potential to modestly influence growth performance; however, it did not lead to positive impacts on carcass characteristics.

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Table 4.1. Diet composition (as-fed basis), Exp. 1[1]

	BW range, kg				
Item:	27 to 45	45 to 61	61 to 77	77 to 104	104 to 127
Ingredient, %					
Corn	56.00	61.25	65.80	69.25	67.25
Soybean meal, 46.5% CP	21.65	16.50	12.00	8.55	20.65
DDGS[2]	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	1.25	1.28	1.23	1.20	1.03
Monocalcium phosphate, 21% P	0.15	---	---	---	0.10
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.36	0.37	0.39	0.39	0.28
DL-Met	0.01	---	---	---	0.04
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	---	0.01	0.02	0.02	---
Ractopamine HCl[3]	---	---	---	---	0.03
Phytase[4]	0.01	0.01	0.01	0.01	0.01
Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[6]	0.08	0.08	0.08	0.08	0.08
Cr[7]	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100
Calculated analysis[8]					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.02	0.91	0.82	0.74	0.90
Ile:Lys	63	62	60	59	64
Leu:Lys	152	159	164	171	150
Met:Lys	29	29	30	31	32
Met and Cys:Lys	55	56	57	59	59
Thr:Lys	61	61	61	63	65
Trp:Lys	18.4	18.4	18.4	18.4	19.0
Val:Lys	70	70	70	70	71
Total Lys, %	1.19	1.06	0.96	0.87	1.04
ME, kcal/kg	3,311	3,320	3,327	3,331	3,320
NE, kcal/kg	2,429	2,465	2,491	2,513	2,476
SID Lys:ME, g/Mcal	3.08	2.74	2.46	2.22	2.71
SID Lys:NE, g/Mcal	4.20	3.69	3.29	2.94	3.64
CP, %	20.0	18.1	16.4	15.1	17.6
Ca, %	0.61	0.57	0.54	0.52	0.50
P, %	0.45	0.40	0.38	0.36	0.40
STTD P, %	0.33	0.29	0.28	0.27	0.29

[1] Treatment diets were fed to 1,206 pigs (PIC, Hendersonville, TN; 337 × 1050, initial BW 28.7 kg] for 125-d in a 5-phase feeding program with 27 pigs per pen and 9 replications per treatment.

[2] DDGS = dried distillers grains with solubles.

[3] Paylean (Elanco, Greenfield, IN).

[4] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.10% STTD P.

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

[6] Provided per kg of premix: 7,054,798 IU vitamin A; 1,102,312 IU vitamin D3; 35,242 IU vitamin E; 3,528 mg vitamin K; 26.5 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; and 6,173 mg riboflavin.

[7] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0.25 kg/tonne (100 µg/kg Cr) or 0.5 kg/tonne (200 µg/kg Cr) at the expense of corn.

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

Table 4.2. Diet composition (as-fed basis), Exp. 2[1]

	BW range, kg			
Item:	45 to 68	68 to 91	91 to 109	109 to 127
Ingredient, %				
Corn	62.76	67.86	70.89	79.71
Soybean meal, 46.5% CP	14.99	9.91	6.90	8.22
DDGS[2]	20.00	20.00	20.00	10.00
Calcium carbonate	1.28	1.23	1.20	1.03
Monocalcium phosphate, 21% P	- - -	- - -	- - -	0.10
Salt	0.35	0.35	0.35	0.35
L-Lys HCl	0.39	0.40	0.40	0.33
L-Thr	0.04	0.05	0.06	0.07
L-Trp	0.01	0.02	0.02	0.01
Phytase[3]	0.01	0.01	0.01	0.01
Trace mineral premix[4]	0.10	0.10	0.10	0.10
Vitamin premix[5]	0.08	0.08	0.08	0.08
Cr[6]	+/-	+/-	+/-	+/-
Total	100	100	100	100
Calculated analysis[7]				
Standardized ileal digestible (SID) amino acids, %				
Lys	0.89	0.78	0.71	0.65
Ile:Lys	60	59	58	58
Leu:Lys	158	166	173	166
Met:Lys	29	30	31	30
Met and Cys:Lys	56	58	60	59
Thr:Lys	60	61	63	65
Trp:Lys	18.0	18.0	18.0	18.0
Val:Lys	69	69	70	69
Total Lys, %	1.04	0.92	0.84	0.76
ME, kcal/kg	3,322	3,329	3,333	3,333
NE, kcal/kg	2,474	2,504	2,522	2,549
SID Lys:ME, g/Mcal	2.68	2.34	2.13	1.95
SID Lys:NE, g/Mcal	3.60	3.11	2.81	2.55
CP, %	17.5	15.6	14.4	12.9
Ca, %	0.57	0.53	0.52	0.46
P, %	0.39	0.37	0.35	0.35
STTD P, %	0.33	0.28	0.27	0.26

[1] Treatment diets were fed to 1,206 pigs (PIC; Hendersonville, TN, 337 × 1050, initial BW 48.9 kg] for 84-d in a 4-phase feeding program with 27 pigs per pen and 15 replications per treatment.

[2] DDGS = dried distillers grains with solubles.

[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.10% STTD P.

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

[5] Premix provided per kg of premix: 7,054,798 IU vitamin A; 1,102,312 IU vitamin D3; 35,242 IU vitamin E; 3,528 mg vitamin K; 26.5 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; and 6,173 mg riboflavin.

[6] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0 or 0.5 kg/tonne (200 µg/kg added Cr) during dietary phase 1 and 2, and 0, 0.25 (100 µg/kg added Cr) or 0.5 kg/tonne (200 µg/kg added Cr) during dietary phase 3 and 4 at the expense of corn.

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

Table 4.3. Chemical analysis of diets, Exp.1 (as-fed basis)[1]

	Added Cr, µg/kg[2]		
BW range, kg	0	100	200
27 to 45			
DM, %	88.1	88.7	88.5
CP, %	19.4	17.9	20.0
Ether extract, %	3.1	2.9	3.4
Crude fiber, %	3.3	3.1	3.3
Cr, µg/kg	590	600	790
45 to 61			
DM, %	85.1	89.0	89.0
CP, %	18.8	15.3	20.2
Ether extract, %	4.6	3.6	3.6
Crude fiber, %	3.2	3.1	3.7
Cr, µg/kg	540	610	710
61 to 77			
DM, %	88.6	88.6	88.7
CP, %	19.5	16.9	15.2
Ether extract, %	3.6	3.8	3.7
Crude fiber, %	3.4	3.1	3.2
Cr, µg/kg	500	430	590
77 to 104			
DM, %	88.7	88.2	89.1
CP, %	15.1	14.5	14.1
Ether extract, %	3.8	3.9	3.8
Crude fiber, %	3.0	3.0	3.2
Cr, µg/kg	480	490	620
104 to 127			
DM, %	88.9	88.3	88.7
CP, %	17.3	16.6	17.7
Ether extract, %	3.1	3.0	2.9
Crude fiber, %	2.6	2.6	3.0
Cr, µg/kg	430	480	610

[1] A composite sample was collected from feeders within treatment and phase, subsampled, and submitted (Ward Laboratories Inc., Kearney, NE) for proximate analysis and to the University of Guelph Agriculture & Food Laboratory (Guelph, ON) for Cr analysis.

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0.25 kg/tonne (100 µg/kg Cr) or 0.5 kg/tonne (200 µg/kg Cr) at the expense of corn.

Table 4.4. Chemical analysis of diets, Exp.2 (as-fed basis)[1]

	Added Cr, µg/kg[2]		
BW range, kg	0	100	200
45 to 68			
DM, %	90.7	- - -	90.9
CP, %	18.1	- - -	18.7
Ether extract, %	3.5	- - -	3.4
Crude fiber, %	1.5	- - -	3.8
Cr, µg/kg	330	- - -	440
68 to 91			
DM, %	90.8	- - -	90.6
CP, %	15.9	- - -	16.1
Ether extract, %	3.7	- - -	3.7
Crude fiber, %	3.7	- - -	3.8
Cr, µg/kg	280	- - -	310
91 to 109			
DM, %	90.9	90.8	90.8
CP, %	15.2	14.9	15.5
Ether extract, %	3.8	4.0	3.7
Crude fiber, %	3.6	3.5	3.6
Cr, µg/kg	290	390	510
109 to 127			
DM, %	90.7	90.9	91.0
CP, %	13.6	16.5	14.9
Ether extract, %	3.3	3.9	3.5
Crude fiber, %	3.0	3.5	3.3
Cr, µg/kg	480	640	680

[1] A composite sample was collected from feeders within treatment and phase, subsampled, and submitted (Ward Laboratories, Inc., Kearney, NE) for proximate analysis and to the University of Guelph Agriculture & Food Laboratory (Guelph, ON) for Cr analysis.

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0 or 0.5 kg/tonne (200 µg/kg added Cr) during dietary phase 1 and 2, and 0, 0.25 (100 µg/kg added Cr) or 0.5 kg/tonne (200 µg/kg added Cr) during dietary phase 3 and 4 at the expense of corn.

Table 4.5. Effects of added Cr-propionate on finishing pig growth and carcass characteristics, Exp. 1[1,2]

							Probability, $P <$	
Grower added Cr, µg/kg:	0	100	200	100	200	SEM	Linear[3]	Quadratic[3]
Finisher added Cr, µg/kg:	0	100	200	200	100			
BW, kg								
Initial	28.7	28.6	28.7	28.6	28.7	0.47	0.955	0.720
End grower	63.5	63.4	61.3	64.1	60.8	0.71	0.001	0.006
Final	139.0	139.9	138.7	140.2	139.4	1.36	0.824	0.354
Grower[4]								
ADG, kg	0.89	0.89	0.83	0.91	0.82	0.012	< 0.001	< 0.001
ADFI, kg	1.77	1.77	1.75	1.78	1.74	0.028	0.229	0.341
G:F	0.50	0.50	0.48	0.51	0.47	0.006	< 0.001	0.001
Finisher[5]								
ADG, kg	0.89	0.91	0.91	0.89	0.93	0.011	0.157	0.019
ADFI, kg	2.45	2.42	2.44	2.44	2.46	0.045	0.656	0.860
G:F	0.36	0.37	0.38	0.37	0.38	0.005	0.015	0.001
Overall								
ADG, kg	0.89	0.90	0.89	0.90	0.89	0.009	0.796	0.136
ADFI, kg	2.23	2.21	2.21	2.23	2.23	0.037	0.472	0.651
G:F	0.40	0.41	0.40	0.40	0.40	0.004	0.463	0.020
Carcass characteristics[6]								
HCW, kg	101.7	102.6	100.9	102.4	101.7	0.92	0.370	0.115
Backfat, mm	16.27	16.32	16.20	16.37	16.23	0.580	0.870	0.805
Lean, %	57.34	57.41	57.44	57.37	57.47	0.406	0.702	0.939
Loin depth, mm	69.98	70.88	70.54	70.80	70.90	0.738	0.503	0.394
Yield, %[7]	73.2	73.3	72.8	73.1	73.0	0.24	0.234	0.370

[1] A total of 1,206 finisher pigs (PIC, Hendersonville, TN; 337 × 1050, initial BW 28.7 kg) were used in a 125-d study with a 5-phase feeding program with 27 pigs per pen and 9 replications per treatment.

[2] Treatment diets were fed in two growth stages, grower (dietary phase 1 and 2) and finisher (dietary phase 3 to 5) and contained 0, 100, or 200 µg/kg Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

[3] Linear and quadratic effects of increasing Cr within the grower and finisher periods were evaluated, as well as linear and quadratic effect of added Cr for treatments at the same level for the full experiment. Additionally, a contrast was constructed comparing the overall growth performance between the two treatments supplemented with 100/200 and 200/100 during the grower and finisher periods, respectively, with no evidence of a difference ($P \geq 0.416$) among treatments in overall growth performance or carcass characteristics.

[4] Dietary phase 1 and 2 fed from d-0 to 39.

[5] Dietary phase 3 to 5 fed from d-39 to 125.

[6] Carcass characteristics other than yield were adjusted to a common HCW by using HCW as a covariate in the statistical model.

[7] Yield was calculated by dividing average pen HCW by average pen live weight collected at the research barn prior to transport to processing facility.

Table 4.6. Effects of Cr-propionate inclusion and feeding duration on finishing pig growth performance and carcass characteristics, Exp. 2[1,2]

					Probability, $P <$			
						Grower	Finisher	
Grower added Cr, µg/kg:	0	200	200	SEM	Overall	0 vs 200	Linear	Quadratic
Finisher added Cr, µg/kg:	0	100	200					
BW, kg								
Initial	48.9	48.9	49.0	0.51	0.840	- - -	- - -	- - -
End grower	91.2	91.5	91.8	0.55	- - -	0.275	- - -	- - -
Final	123.6	123.6	124.6	0.64	0.304	- - -	- - -	- - -
Grower[3]								
ADG, kg	0.88	0.88	0.89	0.007	- - -	0.197	- - -	- - -
ADFI, kg	2.40	2.44	2.42	0.022	- - -	0.239	- - -	- - -
G:F	0.37	0.36	0.37	0.003	- - -	0.861	- - -	- - -
Finisher[4]								
ADG, kg	0.92	0.92	0.94	0.010	- - -	- - -	0.061	0.165
ADFI, kg	2.96	2.94	2.99	0.025	- - -	- - -	0.399	0.201
G:F	0.31	0.31	0.32	0.003	- - -	- - -	0.157	0.731
Overall								
ADG, kg	0.89[b]	0.90[a,b]	0.91[a]	0.006	0.086	- - -	- - -	- - -
ADFI, kg	2.63	2.64	2.66	0.021	0.650	- - -	- - -	- - -
G:F	0.34	0.34	0.34	0.003	0.523	- - -	- - -	- - -
Carcass characteristics[5]								
HCW, kg	95.3	95.3	95.7	0.52	0.741	- - -	- - -	- - -
Loin depth, mm	62.54	63.28	62.86	0.516	0.590	- - -	- - -	- - -
Backfat, mm	18.43	18.03	18.64	0.273	0.229	- - -	- - -	- - -
Lean, %	55.13	55.44	55.03	0.168	0.206	- - -	- - -	- - -
Yield, %[6]	77.1[a]	77.1[a]	76.8[b]	0.10	0.018	- - -	- - -	- - -

[1] A total of 1,206 pigs (PIC, Hendersonville, TN, 337 × 1050, initial BW 48.9 kg) were used in an 84-d study with a 4-phase feeding program with 27 pigs per pen and 15 replications per treatment.

[2] Cr (Chromium propionate; Kemin Industries Inc., Des Moines, IA).

[3] Dietary phase 1 and 2 fed from d-0 to 48.

[4] Dietary phase 3 and 4 fed from d-48 to 84.

[5] Carcass characteristics other than yield were adjusted to a common HCW by using HCW as a covariate in the statistical model.

[6] Yield was calculated by dividing average pen HCW by average pen live weight collected at the research barn prior to transport to processing facility.

[abc] Means lacking common superscripts differ ($P < 0.05$).

Chapter 5 - Determining the influence of chromium propionate and Yucca schidigera on growth performance and carcass composition of pigs housed in a commercial environment[5]

ABSTRACT

Two experiments were conducted to determine the effects of feeding chromium propionate (Cr; Kemin Industries Inc., Des Moines, IA) and a *Yucca schidigera*-based extract (YS; DPI Global, Porterville, CA) on growth performance of finishing pigs housed in commercial conditions. In Exp.1, a total of 1,188 pigs (PIC 337 × 1050; initially 27.3 ± 0.48 kg BW) with 27 pigs per pen and 11 pens per treatment were split by sex upon arrival at the facility, and were randomly allotted to groups of 4 pens blocked by BW. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in 5 phases. Treatments were arranged as a 2 × 2 factorial with main effects of Cr (0 vs. 200 μg/kg) or YS (0 vs. 62.5 mg/kg YS-based feed grade concentrate). Overall, adding Cr alone increased ($P = 0.049$) ADFI, and inclusion of YS resulted in a marginally significant increase ($P = 0.077$) in ADFI. Backfat depth was increased ($P = 0.043$) and lean percentage was decreased ($P = 0.011$) with added Cr. In Exp.2, a total of 2,430 pigs (PIC 359 × 1050; initially 29.3 ± 0.43 kg BW) were placed in balanced mixed-sex pens with 27 pigs per pen, blocked by average pen BW, and randomly assigned to 1 of 6 dietary treatments with 14 pens per treatment. Diets were corn-soybean meal-based and were formulated in 5 dietary phases. Treatments were arranged in a 2 × 3 factorial with main

[5] This work has been published in *Translational Animal Science*: J. T. Gebhardt, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. A. Loughmiller, A. L. P. de Souza, M. J. Rincker, and S. S. Dritz. 2019. Determining the influence of chromium propionate and Yucca schidigera on growth performance and carcass composition of pigs housed in a commercial environment. Transl. Anim. Sci. 3:1275-1285. doi:10.1093/tas/txz117.

effects of Cr (0 vs. 200 µg/kg added Cr), and YS extract (0, 62.5, or 125 mg/kg YS-based feed grade concentrate). Overall, a marginally significant (linear; $P = 0.072$) Cr × YS interaction was observed for ADG where there was insufficient evidence of a difference with increasing YS in diets not including added Cr ($P \geq 0.109$), however ADG increased (quadratic, $P = 0.026$) with YS addition in treatments fed 200 µg/kg added Cr. For overall ADFI, a marginally significant (linear, $P = 0.071$) Cr × YS interaction was observed where YS increased ADFI with 200 µg/kg added Cr (linear, $P = 0.031$), however did not when diets contained no added Cr ($P = 0.700$). A marginally significant reduction in G:F was observed when 62.5 mg/kg YS was included (quadratic, $P = 0.053$), and final BW and HCW were lowest with 62.5 mg/kg YS (quadratic; $P = 0.012$). In summary, adding Cr propionate along with YS led to modest changes in performance with the greatest benefit observed with 200 µg/kg Cr and 125 mg/kg YS-based feed grade concentrate.

Keywords: chromium propionate, finishing pigs, *Yucca schidigera*

INTRODUCTION

Chromium (Cr) supplementation in swine feeding programs has been evaluated with significant variability in observed responses. Chromium plays a role in the metabolism of multiple molecules including carbohydrates, lipids, proteins, and nucleic acids (NRC, 2012). Corn-soybean meal-based diets contain a significant amount of Cr ranging from 750 to 3,000 µg/kg, but much of that is thought to be unavailable to the animal (NRC, 2012). Such variability in composition in ingredients may be one of the factors leading to significant variability in the presence and magnitude of response in swine. A meta-analysis was conducted by Sales and Jancik (2011) and observed that dietary supplementation with Cr to be associated with improved carcass characteristics including reduced backfat and increased percentage lean along with

improved growth performance. Additionally, *Yucca schidigera* (YS) is believed to have a positive impact on gastrointestinal microflora (Katsunuma et al., 2000) through its saponin characteristics, thereby reducing gaseous emissions (Sun et at., 2017) and potentially improving growth performance. Supplementation of YS in finishing pig diets has shown improvements in finishing pig G:F (Mader and Brumm, 1987); however, several additional studies in both nursery and finishing pigs did not observe a benefit (Amon et al., 1995; Van den Berghel et al., 2000; Colina et al., 2001, Panetta et al., 2006). Research related to the impact of YS on blood metabolites in swine is currently limited; however, has been shown to reduce cholesterol and triglyceride levels (Munoz et al., 2008) in swine. Currently, no evidence exists evaluating potential interactions between Cr and YS-based feed additives. Therefore, the objective of this series of experiments was to further determine the effects of Cr supplementation with and without a YS-based feed grade concentrate supplemented at multiple levels on growth performance and carcass composition of pigs housed in a commercial environment.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. The studies were conducted at a commercial research-finishing site in southwest Minnesota using three identical barns. The barns were naturally ventilated and double-curtain-sided. Each pen (5.5 × 3.0 m) was equipped with a 4-hole stainless steel feeder and cup waterer for ad libitum access to feed and water and allowed approximately 0.61 m^2/pig. Feed additions to each pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN).

Animals and Diets

In Exp. 1, a total of 1,188 pigs [PIC (Hendersonville, TN) 337 × 1050, initially 27.3 ± 0.48 kg BW] with 27 pigs per pen and 11 pens per treatment were used. Pigs were split by sex upon arrival at the facility, with 5 blocks of each sex and a final mixed sex block. Blocks were randomly allotted to groups of 4 pen locations within the barn and randomized to dietary treatment within block. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in 5 phases. All diets were formulated to meet or exceed NRC (2012) requirement estimates (Table 1). Treatments were arranged in a 2 × 2 factorial with main effects of Cr (0 vs. 200 μg/kg; KemTRACE Chromium; Kemin Industries Inc., Des Moines, IA) or YS (0 vs. 62.5 mg/kg; Micro-Aid; Distributors Processing Inc., Porterville, CA) and were fed for the full duration of the experiment. Ractopamine HCl (Paylean 19.84 g/kg; Elanco Animal Health, Greenfield, IN) was included in phase 5 diets and fed for 27-d. Experimental diets were manufactured at a commercial feedmill (New Horizon Feeds, Pipestone, MN). The pigs used in the current experiments did not experience significant health challenges.

In Exp. 2, a total of 2,430 pigs [PIC (Hendersonville, TN) 359 × 1050, initially 29.3 ± 0.43 kg BW] with 27 pigs per pen and 14 pens per treatment were used. Pigs were placed in two research barns on separate weaning dates, each filling independently. Pigs were placed in mixed-sex pens with equal numbers of barrows and gilts across pens, blocked by average pen BW within barn, and randomly assigned to treatment. Diets were corn-soybean meal-based and formulated in 5 dietary phases to meet or exceed NRC (2012) requirement estimates within phase. Dietary treatments were fed for the full duration of the study and arranged in a 2 × 3 factorial. Main effects included added Cr (0 vs. 200 μg/kg), and YS-based feed grade concentrate

(0, 62.5, or 125 mg/kg). All diets were manufactured at a commercial feed mill (New Horizon Feeds, Pipestone, MN; Table 2) and fed in meal form. No ractopamine HCl was used in Exp. 2.

In both experiments, pens of pigs were weighed and feeder measurements were recorded a minimum of every 14-d and at dietary phase changes, first marketing, and conclusion of the trial to determine ADG, ADFI, and G:F. The 3 largest pigs/pen were selected using visual evaluation by trained personnel and marketed at an average barn weight (Exp 1: 111 kg on d-97; Exp. 2: 118 kg on d-95 for barn 1, 113 kg on d-98 for barn 2) following the routine farm protocol with no carcass data collected on this subset. At the conclusion of the trial (Exp. 1, d-117; Exp. 2, d-103 for barn 1, d-113 for barn 2), the remaining animals were given a tattoo corresponding to pen number and were transported to a commercial packing facility (JBS Swift and Company; Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included live pen weight, HCW, backfat, percentage carcass lean, and loin depth. Loin depth and backfat were measured using an optical probe inserted between the third and fourth ribs from the caudal aspect of the carcass at a distance approximately 7 cm from dorsal midline as described by Gebhardt et al. (2018). Percentage carcass lean was calculated using a proprietary formula using HCW, backfat depth, and loin depth. Additionally, percentage yield was calculated by dividing pen average HCW by pen average live weight collected at the research facilities prior to transport to processing facility. On the morning of scheduled transport to the packing facility, the electronic feeding unit was shut off and no further feed was delivered to pens. Pigs were loaded in late afternoon, transported approximately 70 miles to the packing facility, and were harvested beginning early the following day.

Chemical Analysis

For both experiments, complete diet samples were collected from multiple feeders within treatment, combined within phase when applicable, and subsampled for analysis. All feed samples were analyzed (Ward Laboratories; Kearney, NE) for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), crude fiber (AOAC 978.10, 2006) and control diets (no added Cr, no added YS) were submitted to University of Guelph Agriculture & Food Laboratory (Guelph, ON) for analysis of baseline Cr (US EPA 6020a, 1998).

Statistical Analysis

Data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. For Exp. 1, block was included in the model as a random effect and accounted for gender, location within barn, and initial BW at the time of allotment. For Exp. 2, weight block was included in the model as a random effect, which also accounted for barn. Linear and quadratic interactive effects were evaluated in the statistical model, as well as the main effect of added Cr and linear and quadratic effects of increasing YS. Backfat, loin depth, and percentage lean were adjusted to a common carcass weight using HCW as a covariate. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Chemical analysis of diets fed in Exp. 1 (Table 3) and Exp. 2 (Table 4) resulted in no notable differences among treatments. Chromium analysis yielded a significant amount of variability among control diets across dietary phase and experiment, ranging from 450 to 1,700

μg/kg. Analyzed Cr in control diets in Exp. 1 averaged across dietary phases was 570 μg/kg, and for Exp. 2 was 1,270 μg/kg.

Exp. 1

In Exp. 1, there was no evidence of a Cr × YS interaction observed ($P \geq 0.149$) for any growth performance or carcass characteristics (Table 5). For the grower period, added Cr increased ($P \leq 0.028$) ADG and ADFI. Added YS in the grower period resulted in a reduction ($P = 0.050$) in G:F. During the finishing period, added Cr resulted in a marginally significant increase in ADFI ($P = 0.080$) and reduction in G:F ($P = 0.056$). Added YS in the finishing period resulted in a marginally significant increase ($P = 0.088$) in ADFI. Overall, ADG and G:F were not influenced by treatment ($P \geq 0.115$). Overall ADFI was increased ($P = 0.049$) with Cr supplementation, and added YS resulted in a marginally significant improvement ($P = 0.077$) in ADFI. Carcass characteristics including HCW, loin depth, and carcass yield were not influenced by treatment ($P \geq 0.136$). Backfat depth was increased ($P = 0.043$) and lean percentage decreased ($P = 0.011$) when Cr was added to diets.

Exp. 2

In Exp. 2, increasing YS in the grower period resulted in a marginally significant (Table 6; quadratic, $P = 0.093$) decrease then increase in ADG, with the poorest gain observed in pigs fed diets with 62.5 mg/kg YS and the best gain observed in pigs fed 125 mg/kg. This resulted in a marginally significant Cr × YS interaction (linear, $P = 0.100$) for BW at the end of the grower period. Body weight at the end of the grower period was similar across YS treatments when Cr was not included in the diet ($P \geq 0.322$), but was increased in a marginally significant manner when YS increased from 62.5 to 125 mg/kg in diets containing 200 ppb added Cr (quadratic, $P =$

0.053). Inclusion of Cr or YS had no effect ($P \geq 0.105$) on ADFI or G:F during the grower period.

During the finishing phase, pigs fed 62.5 mg/kg YS had decreased (quadratic, $P = 0.018$) ADG compared to control and 125 mg/kg YS-fed pigs. There was no evidence of differences ($P \geq 0.162$) among pigs for ADFI observed within the finishing period. A marginally significant reduction (linear, $P = 0.051$) in G:F was observed in pigs fed increasing YS. Added Cr during the finishing period did not influence ADG, ADFI, or G:F ($P \geq 0.167$).

For the overall data, a marginally significant (linear, $P = 0.072$) Cr × YS interaction was observed for ADG. Sufficient evidence of a linear relationship with increasing YS supplementation within either Cr level was not present ($P \geq 0.193$), however ADG increased (quadratic, $P = 0.026$) in treatments fed 200 µg/kg added Cr. A marginally significant Cr × YS interaction ($P = 0.071$) was observed for overall ADFI where YS inclusion resulted in an increase in ADFI when 200 µg/kg added Cr was fed (linear, $P = 0.031$), however no evidence of a difference was observed in diets containing no added Cr ($P = 0.700$). YS supplementation had a marginally significant impact on overall G:F, with the lowest G:F observed in diets containing 62.5 mg/kg added YS (quadratic, $P = 0.053$). A marginally significant (linear, $P = 0.058$) Cr × YS interaction was observed for final BW. Sufficient evidence of a linear relationship with increasing YS supplementation within either Cr level was not present ($P \geq 0.159$), however final BW increased (quadratic, $P = 0.041$) in treatments fed 200 µg/kg added Cr. The main effect of added YS on final BW (quadratic, $P = 0.012$) resulted in pigs fed diets with 62.5 mg/kg having the lowest final BW. Added Cr alone did not influence overall growth performance ($P \geq 0.299$). Carcass characteristics including HCW, loin depth, backfat, percentage lean, and percentage yield were not influenced by added Cr ($P \geq 0.278$). Inclusion of YS at 62.5 mg/kg resulted in the

lowest HCW (quadratic, $P = 0.012$), but did not influence loin depth, backfat, percentage lean, or percentage yield ($P \geq 0.152$).

DISCUSSION

Chromium interacts in many metabolic pathways as summarized by NRC (2012). Chromium supplementation in swine feeding programs has shown a significant variability in observed response in scientific literature, as briefly summarized by Gebhardt et al. (2018). A number of factors are likely involved with the variability in growth performance benefits, including dietary composition, Cr source, environmental, and management factors (Lindemann, 2007). Chromium is present in natural feedstuffs at relatively low levels ranging from 750 to 3,000 µg/kg (NRC, 2012), with a high degree of variability. Additionally, Cr originating from natural feedstuffs has a low bioavailability (NRC, 2012), and organic forms are believed to be much more bioavailable (Lindemann, 2007). For these reasons, evaluation of dietary Cr content can be an analytical challenge.

Sales and Jancik (2011) attempted to summarize and quantify the effect of dietary Cr supplementation on carcass characteristics and growth performance of finishing swine across 31 studies using meta-analysis. Multiple sources and levels of Cr were included in the analysis, including Cr methionine chelate, Cr nanocomposite, Cr nicotinate, Cr propionate, Cr tripicolinate, and Cr yeast. The analysis suggested that when Cr is included in swine finishing diets, the carcass would have a reduced backfat thickness, increased percentage lean, and increased loin muscle area compared to no Cr supplementation. In Exp. 1, inclusion of Cr propionate resulted in greater backfat and reduced percentage lean, which is in contrast to what Sales and Jancik (2011) observed. Supplementation of Cr had no influence on carcass characteristics in Exp. 2. An important distinction to make when evaluating Sales and Jancik

(2011) is a number of Cr sources, supplementation doses ranging up to 800 μg/kg, and significant variation in the date of publication. Studies included in the meta-analysis varied from 1993 until 2009, and could represent significant differences in genetic lean growth potential compared to modern high-producing genetic potential. Nonetheless, the current series of experiments did not demonstrate a significant improvement in carcass characteristics with Cr supplementation as would be suggested by summary of previously published literature.

In addition to carcass characteristics, Sales and Jancik (2011) found Cr supplementation to be positively correlated with ADG and G:F. In Exp. 1, Cr supplementation improved overall ADFI, however had no influence on overall ADG or G:F. In Exp. 2, marginally significant Cr × YS interactions were present for both ADG and ADFI, where YS supplementation increased performance when 200 μg/kg added Cr was included. In the current series of experiments, overall ADG and G:F were not improved by Cr supplementation with the exception of a marginally significant interaction for overall ADG in Exp. 2. Supplementation of Cr increased ADG during the growing period of Exp. 1; however, no additional impact was observed in finishing or overall ADG.

Research designed to directly evaluate the impact of YS supplementation on growth performance of finishing pigs and carcass characteristics is relatively scarce in recent years, and no previous research has evaluated the combination of YS and Cr propionate under commercial conditions. The saponin characteristics of YS are thought to convert ammonia to less volatile forms such as ammonium nitrogen (Panetta et al., 2006), thereby reducing ammonia concentrations in swine production facilities (Cheeke et al., 2000; Colina et al., 2001), as well as more generally reduce odorants in poultry production (Matusiak et al., 2016). Many such studies evaluating gaseous emissions also report growth performance measurements (Amon et al., 1995;

Colin et al., 2001; Panetta et al., 2006). Within these studies, there was no evidence of differences in growth performance between pigs fed YS-extract and control. However, it is important to consider such studies were not designed with evaluation of growth performance as the primary objective.

In Exp. 1, YS supplementation at 62.5 mg/kg resulted in the lowest G:F in the grower period; however, evidence was not sufficient for any influence on overall G:F. Similarly in Exp. 2, a marginally significant quadratic relationship was observed for G:F as YS supplementation was increased, resulting in 62.5 mg/kg YS having the lowest G:F. Hong et al. (2001) observed a quadratic relationship for finishing pig ADG and ADFI with 0, 60, and 120 mg/kg added YS-based extract where pigs fed 60 mg/kg had the poorest performance. These results are in contrast to Mader and Brumm (1987), which observed an improvement in finishing pig G:F when sarsaponin was supplemented at 63 mg/kg, particularly in late finishing.. Mader and Brumm (1987), however, also found no evidence of a difference in G:F in a separate experiment with sarsaponin supplementation when fed to pigs ranging from 19 to 52 kg. Van den Berghel et al. (2000) found no evidence of a difference in ADG or G:F with YS supplementation to finishing pigs; however, they noted evidence of improved respiratory health as quantified by a reduction in presence of respiratory pathology. A clear understanding of the reduced performance observed with supplementation of 62.5 mg/kg in the current series of experiments is unknown; however, it is in agreement with Hong et al. (2001).

Additional mechanisms by which YS could affect swine growth performance could include impact on cholesterol and triglyceride concentrations as shown by Munoz et al. (2008). Hong et al. (2001) found no evidence of a difference in serum total cholesterol, low-density lipoprotein (LDL), or high-density lipoprotein (HDL) concentrations when 60 or 120 mg/kg YS-

based extract was fed to finishing pigs compared to control. Kucukkurt et al. (2016) observed supplementation of YS-extracts in mice influenced both blood biochemical status including glucose and lipoproteins as well metabolic hormone status. A greater understanding of the impact of YS on lipid metabolism pathways in swine is warranted and necessary. Additional potential mechanisms that have been previously described by which YS may influence growth performance include modulation of gastrointestinal microbiota (Katsunuma et al., 2000) and anti-inflammatory properties via inhibition of NFkappaB and subsequent oxygen free radical scavenging (Cheeke et al., 2006). The objective of the current series of experiments was to evaluate YS in a commercial environment, however further evaluation is warranted on a basic level.

To date, no published literature has evaluated the combined effect of Cr and YS in swine. Previously described mechanisms for both Cr and YS appear to be independent; however, there is some evidence specific to YS mechanisms observed in poultry that may indicate potential for a synergistic effect. Rezaei et al. (2017) found improvement in gain and G:F of broilers under heat stress conditions; however, no impact was observed in thermoneutral conditions. The authors propose the mechanism leading to these observations was through YS-associated antagonism of glucocorticoids produced in stress events. The action of Cr on glucose metabolism is thought to be through increased tissue sensitivity to insulin (NRC, 2012) and subsequent cellular uptake of glucose. In humans, glucocorticoids have been shown to reduce glucose uptake by skeletal muscle cells (Kuo et al., 2015). Furthermore, Lopes et al. (2004) observed that pigs given dexamethasone had greater circulating levels of glucose and insulin. Although evidence in swine is currently lacking, potential may be present for a synergistic relationship leading to improved uptake of glucose, particularly under stressful events when glucocorticoids are elevated. The

current series of experiments were designed to evaluate the combination of YS and Cr in commercial conditions, therefore no substantial stressful conditions were induced and no additional evidence for this hypothesis can be currently derived. There was marginally significant evidence of a Cr × YS interaction for overall ADG and ADFI in Exp. 2, where YS supplementation improved growth performance in the presence of 200 µg/kg added Cr and the impact was not observed when no Cr was added to the diets. Further understanding and evaluation is necessary prior to drawing conclusions regarding potential synergistic effects, and cannot be fully derived at this time due to a complete lack of literature evaluating such a combination.

In conclusion, limited synergistic effects were observed when feeding both Cr and YS to pigs housed in a commercial environment. Significant variability was observed in analysis of Cr content of manufactured control diets, reinforcing the challenges associated with very low inclusion rates and significant variability in ingredient Cr content. Both Cr and YS have potential to elicit slight improvements in growth performance when used in commercial conditions as evaluated in the current series of experiments, however no evidence of improved carcass characteristics was observed.

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Table 5.1. Diet composition, Exp. 1 (as-fed basis)[1]

	BW range, kg				
Item	27 to 45	45 to 61	61 to 77	77 to 104	104 to 127
Ingredient, %					
Corn	56.05	61.25	65.80	69.25	67.25
Soybean meal, 46.5% CP	21.60	16.50	11.95	8.55	20.65
DDGS[2]	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	1.25	1.28	1.23	1.20	1.03
Monocalcium phosphate, 21% P	0.15	---	---	---	0.10
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.36	0.37	0.39	0.39	0.28
DL-Met	0.01	---	---	---	0.04
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	---	0.01	0.02	0.02	---
Ractopamine HCl[3]	---	---	---	---	0.03
Phytase[4]	0.01	0.01	0.01	0.01	0.01
Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[6]	0.08	0.08	0.08	0.08	0.08
Cr[7]	+/-	+/-	+/-	+/-	+/-
Yucca schidigera[8]	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100
Calculated analysis[9]					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.02	0.91	0.82	0.74	0.90
Ile:Lys	63	62	60	59	64
Leu:Lys	152	159	164	171	150
Met:Lys	29	29	30	31	32
Met and Cys:Lys	55	56	57	59	59
Thr:Lys	61	61	61	63	65
Trp:Lys	18.4	18.4	18.4	18.4	19.0
Val:Lys	70	70	70	70	71
Total Lys, %	1.18	1.06	0.96	0.87	1.04
ME, kcal/kg	3,311	3,320	3,327	3,331	3,320
NE, kcal/kg	2,432	2,465	2,491	2,513	2,476
SID Lys:ME, g/Mcal	3.08	2.74	2.46	2.22	2.71
SID Lys:NE, g/Mcal	4.20	3.69	3.29	2.94	3.64
CP, %	20.0	18.1	16.4	15.0	17.6
Ca, %	0.61	0.57	0.54	0.52	0.50
P, %	0.45	0.40	0.38	0.36	0.40
STTD P, %	0.33	0.29	0.28	0.27	0.29

[1] A total of 1,188 finisher pigs [PIC (Hendersonville, TN) 337 × 1050; initial BW = 27.3 ± 0.48 kg] were used in a 117-d, 5-phase finisher study with 27 pigs per pen and 11 replications per treatment.
[2] DDGS = dried distillers grains with solubles.
[3] Paylean 19.84 g/kg (Elanco, Greenfield, IN).
[4] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.10% STTD P.
[5] Premix provided per kg of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 33 g Mn from manganese oxide; 17 g Cu from copper sulfate; 330 mg I from calcium iodate; and 300 mg Se from sodium selenite.
[6] Premix provided per kg of premix: 7,054,798 IU vitamin A; 1,102,312 IU vitamin D3; 35,242 IU vitamin E; 3,528 mg vitamin K; 26.5 mg vitamin B12; 39,683 mg niacin; 22,046 mg pantothenic acid; and 6,173 mg riboflavin.
[7] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0.5 kg/tonne (200 µg/kg Cr) at the expense of corn in the appropriate treatment diets.
[8] *Yucca schidigera*-based feed grade concentrate (Distributors Processing Inc., Porterville, CA) was added at 1 kg/tonne in the appropriate treatment diets at the expense of corn to provide 62.5 mg/kg active ingredient in the completed diet.
[9] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, D.C.

Table 5.2. Diet composition, Exp. 2 (as-fed basis)[1]

	BW range, kg				
Item	27 to 45	45 to 68	68 to 91	91 to 109	109 to 127
Ingredient, %					
Corn	55.04	63.03	68.13	71.29	79.82
Soybean meal, 46.5% CP	22.80	14.97	9.89	6.71	8.21
DDGS[2]	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	0.95	0.98	0.90	0.93	0.85
Monocalcium phosphate, 21% P	0.25	0.08	0.10	0.08	0.20
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.38	0.39	0.40	0.41	0.33
DL-Met	0.02	- - -	- - -	- - -	- - -
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	- - -	0.01	0.02	0.02	0.01
Phytase[3]	0.01	0.01	0.01	0.01	0.01
Vitamin/trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15
Cr[5]	+/-	+/-	+/-	+/-	+/-
Yucca schidigera[6]	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100
Calculated analysis[7]					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.06	0.89	0.78	0.71	0.65
Ile:Lys	62	60	59	58	58
Leu:Lys	149	158	166	173	166
Met:Lys	30	29	30	31	30
Met and Cys:Lys	55	56	58	60	59
Thr:Lys	61	60	61	63	65
Trp:Lys	18.3	18.1	18.0	18.0	18.0
Val:Lys	69	69	69	69	69
Total Lys, %	1.23	1.04	0.92	0.84	0.76
ME, kcal/kg	3,318	3,331	3,338	3,342	3,338
NE, kcal/kg	2,429	2,480	2,511	2,531	2,553
SID Lys:ME, g/Mcal	3.19	2.67	2.34	2.12	1.95
SID Lys:NE, g/Mcal	4.36	3.59	3.11	2.81	2.55
CP, %	20.5	17.5	15.6	14.4	12.9
Ca, %	0.52	0.48	0.43	0.43	0.42
P, %	0.48	0.41	0.39	0.37	0.37
STTD P, %	0.35	0.30	0.29	0.28	0.28

[1] A total of 2,430 finisher pigs [PIC (Hendersonville, TN) 359 × 1050; initial BW = 29.3 ± 0.43 kg] were used in a 5-phase finisher study with 27 pigs per pen and 14 replications per treatment.

[2] DDGS = dried distillers grains with solubles.

[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.10% STTD P.

[4] Premix provided per kg of premix: 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 220 mg I from calcium iodate; and 200 mg Se from sodium selenite, 3,527,399 IU vitamin A; 881,850 IU vitamin D3; 17,637 IU vitamin E; 1,766 mg vitamin K; 15 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; and 3,307 mg riboflavin.

[5] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0 or 0.5 kg/tonne (200 µg/kg added Cr) at the expense of corn.

[6] *Yucca schidigera*-based feed grade concentrate (Distributor's Processing, Inc., Porterville, CA) was added at 0, 1, or 2 kg/tonne at the expense of corn to provide 0, 62.5, and 125 mg/kg active ingredient in the completed diets, respectively.
[7] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, D.C.

Table 5.3. Chemical analysis of diets, Exp. 1 (as-fed basis)[1]

Added Cr, µg/kg[2]:	0	200	0	200
Yucca schidigera, mg/kg[3]:	0	0	62.5	62.5
BW range, kg				
27 to 45				
DM, %	88.0	87.1	88.7	88.3
CP, %	19.9	17.8	20.0	18.5
Ether extract, %	3.7	3.2	3.6	3.5
Crude fiber, %	3.6	3.6	3.0	2.8
45 to 61				
DM, %	88.1	88.8	88.2	87.4
CP, %	16.8	18.2	21.4	19.2
Ether extract, %	4.3	3.6	3.7	3.3
Crude fiber, %	3.4	3.5	3.5	4.6
61 to 77				
DM, %	88.9	88.7	88.5	89.3
CP, %	16.7	16.2	16.9	15.5
Ether extract, %	3.8	3.8	3.8	3.7
Crude fiber, %	3.2	3.0	3.3	3.1
77 to 104				
DM, %	88.3	88.3	88.6	88.7
CP, %	15.1	15.2	15.4	14.9
Ether extract, %	3.7	3.6	3.8	3.6
Crude fiber, %	3.1	3.2	3.1	2.9
104 to 127				
DM, %	87.6	88.9	88.7	88.3
CP, %	17.6	17.8	17.5	19.5
Ether extract, %	3.0	3.2	3.1	3.0
Crude fiber, %	2.6	2.9	2.8	2.8

[1] A composite sample was collected from feeders within treatment and phase, subsampled, and submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0.5 kg/tonne (200 µg/kg Cr) at the expense of corn in the appropriate treatment diets.

[3] *Yucca schidigera*-based feed grade concentrate (Distributors Processing Inc., Porterville, CA) was added at 1 kg/tonne (62.5 mg/kg active ingredient) at the expense of corn in the appropriate treatment diets.

Table 5.4. Chemical analysis of diets, Exp. 2 (as-fed basis)[1,2]

Added Cr, µg/kg[2]:	0	0	0	200	200	200
Yucca schidigera, mg/kg[3]:	0	62.5	125	0	62.5	125
BW range, kg						
27 to 45						
DM, %	89.8	89.4	89.7	89.9	89.7	89.6
CP, %	20.7	21.7	21.6	21.3	20.6	21.5
Ether extract, %	3.4	3.7	3.4	3.3	3.6	3.7
Crude fiber, %	2.1	2.4	2.5	2.0	2.1	2.6
45 to 68						
DM, %	89.7	89.9	90.0	89.7	90.3	89.7
CP, %	19.2	18.7	19.7	19.5	19.6	19.1
Ether extract, %	3.8	3.6	3.6	3.6	3.9	3.7
Crude fiber, %	2.1	2.1	2.9	2.8	3.4	2.8
68 to 91						
DM, %	89.6	89.4	89.3	89.0	88.9	89.7
CP, %	16.2	16.1	16.0	16.1	15.0	15.6
Ether extract, %	3.8	3.7	4.0	3.9	4.0	3.9
Crude fiber, %	2.5	2.7	2.9	2.6	2.6	2.4
91 to 109						
DM, %	89.4	90.0	89.8	89.0	89.6	89.6
CP, %	16.8	17.7	18.4	15.9	15.7	17.9
Ether extract, %	3.9	3.6	3.7	3.8	3.9	3.8
Crude fiber, %	2.5	3.3	3.3	3.2	2.1	2.5
109 to 127						
DM, %	88.8	90.4	89.0	89.9	89.2	88.9
CP, %	13.9	13.8	14.2	12.7	13.9	14.9
Ether extract, %	3.5	3.8	4.0	3.6	4.0	4.2
Crude fiber, %	2.4	1.9	2.4	1.8	2.6	2.4

[1] A composite sample was collected from feeders within treatment and phase, subsampled, and submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0 or 0.5 kg/tonne (200 µg/kg added Cr) at the expense of corn.

[3] *Yucca schidigera*-based feed grade concentrate (Distributor's Processing, Inc., Porterville, CA) was added at 0, 1, or 2 kg/tonne at the expense of corn to provide 0, 62.5, and 125 mg/kg active ingredient, respectively.

Table 5.5. Impact of Cr and *Yucca schidigera* on finishing pig growth performance and carcass characteristics, Exp. 1[1]

						Probability, $P <$		
Added Cr, µg/kg:[2]	0	200	0	200		*Yucca*		*Yucca*
Yucca schidigera, mg/kg:[3]	0	0	62.5	62.5	SEM	*schidigera* × Cr	Cr	*schidigera*
BW, kg								
Initial	27.3	27.3	27.3	27.4	0.48	0.653	0.907	0.726
End grower	60.5	61.0	60.4	61.0	0.79	0.906	0.099	0.916
Final	129.2	129.7	129.5	130.4	1.30	0.893	0.472	0.609
Grower[4]								
ADG, kg	0.85	0.86	0.85	0.86	0.010	0.989	0.027	0.713
ADFI, kg	1.73	1.77	1.75	1.78	0.033	0.980	0.028	0.224
G:F	0.49	0.49	0.48	0.48	0.005	0.975	0.769	0.050
Finisher[5]								
ADG, kg	0.88	0.88	0.89	0.89	0.010	0.761	0.954	0.334
ADFI, kg	2.47	2.52	2.52	2.56	0.056	0.907	0.080	0.088
G:F	0.36	0.35	0.35	0.35	0.006	0.924	0.056	0.418
Overall								
ADG, kg	0.87	0.87	0.87	0.88	0.009	0.810	0.446	0.490
ADFI, kg	2.22	2.26	2.26	2.30	0.047	0.955	0.049	0.077
G:F	0.39	0.39	0.39	0.38	0.006	0.915	0.115	0.178
Carcass characteristics[6]								
HCW, kg	96.2	97.6	97.5	98.3	1.11	0.716	0.136	0.165
Backfat, mm	16.87	17.83	17.26	17.43	0.269	0.149	0.043	0.993
Lean, %	56.98	56.41	56.83	56.51	0.446	0.457	0.011	0.880
Loin depth, mm	70.22	70.03	70.71	69.56	0.668	0.312	0.163	0.987
Yield, %[7]	74.50	75.22	75.28	75.43	0.456	0.510	0.305	0.251

[1] A total of 1,188 finisher pigs [PIC (Hendersonville, TN) 337 × 1050; initial BW = 27.3 ± 0.48 kg] were used in a 117-d, five phase finisher study with 27 pigs per pen and 11 replications per treatment.

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

[3] *Yucca schidigera*-based feed grade concentrate (Distributors Processing, Inc., Porterville, CA).

[4] Dietary phase 1 and 2 fed from d-0 to 39.

[5] Dietary phase 3 to 5 fed from d-39 to 117.

[6] The largest 3 pigs were marketed from each pen on d-97. All remaining pigs were marketed from each pen on d-117. Carcass characteristics other than yield were adjusted to a common HCW by inclusion of HCW as a covariate in the statistical model.

[7] Yield was calculated by dividing HCW by average pen live weight collected at the research barn prior to transport to processing facility.

Table 5.6. Impact of Cr and *Yucca schidigera* inclusion on finishing pig growth performance and carcass characteristics, Exp. 2[1]

								Probability, $P <$				
								Yucca schidigera × Cr			*Yucca schidigera*	
Added Cr, µg/kg:[2]	0	0	0	200	200	200	SEM	Linear	Quad	Cr	Linear	Quad
Yucca schidigera, mg/kg:[3]	0	62.5	125	0	62.5	125						
BW, kg												
Initial	29.3	29.3	29.3	29.3	29.3	29.3	0.43	0.902	0.679	0.991	0.965	0.885
End grower[4]	91.4	91.0	90.8	91.0	90.4	91.8	0.92	0.100	0.254	0.971	0.797	0.109
Final[5]	124.2	122.5	123.1	123.9	123.0	124.9	0.84	0.058	0.723	0.167	0.936	0.012
Grower[6]												
ADG, kg	0.90	0.89	0.89	0.89	0.88	0.90	0.009	0.166	0.265	0.945	0.682	0.093
ADFI, kg	2.19	2.19	2.18	2.17	2.18	2.21	0.023	0.105	0.656	0.936	0.448	0.734
G:F	0.41	0.41	0.41	0.41	0.41	0.41	0.006	0.540	0.578	0.958	0.652	0.138
Finisher[7]												
ADG, kg	0.89	0.86	0.87	0.88	0.87	0.89	0.022	0.237	0.576	0.167	0.509	0.018
ADFI, kg	2.97	2.92	2.96	2.93	2.96	2.99	0.037	0.162	0.326	0.601	0.232	0.252
G:F	0.30	0.29	0.29	0.30	0.29	0.30	0.005	0.887	0.762	0.236	0.051	0.092
Overall												
ADG, kg[8]	0.89	0.88	0.88	0.89	0.88	0.90	0.013	0.072	0.649	0.299	0.976	0.007
ADFI, kg[9]	2.46	2.44	2.45	2.43	2.45	2.48	0.019	0.071	0.783	0.686	0.202	0.377
G:F	0.36	0.36	0.36	0.37	0.36	0.36	0.006	0.673	0.444	0.516	0.119	0.053
Carcass characteristics[10]												
HCW, kg	93.3	91.9	92.8	92.9	92.5	93.7	1.05	0.183	0.668	0.315	0.774	0.012
Loin depth, mm	66.39	65.85	66.40	66.42	65.94	65.16	0.784	0.196	0.422	0.353	0.206	0.648
Backfat, mm	16.56	16.25	16.45	16.69	16.36	16.69	0.292	0.811	0.851	0.409	0.819	0.152
Lean, %	56.73	56.85	56.79	56.65	56.79	56.50	0.226	0.482	0.642	0.278	0.787	0.267
Yield, %[11]	75.2	75.0	75.4	75.0	75.2	75.0	0.74	0.472	0.155	0.426	0.568	0.542

[1] A total of 2,430 finisher pigs [PIC (Hendersonville, TN) 359 × 1050; initial BW = 29.3 ± 0.43 kg] were used in a 5-phase finisher study with 27 pigs per pen and 14 replications per treatment using two commercial grow-finish barns (hereafter described as barn 1 and barn 2).

[2] Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA) was added at 0 or 0.50 kg/tonne (200 µg/kg added Cr) at the expense of corn.

[3] *Yucca schidigera*-based feed grade concentrate (YS; Distributor's Processing, Inc.) was added at 0, 1, or 2 kg/tonne at the expense of corn to provide 0, 62.5, and 125 mg/kg *yucca schidigera*, respectively.
[4] Dose effect of YS when Cr not included in diet, linear, $P = 0.322$, quadratic, $P = 0.743$; Dose effect of YS when Cr included in diet, linear, $P = 0.179$, quadratic, $P = 0.053$.
[5] Dose effect of YS when Cr not included in diet, linear, $P = 0.159$, quadratic, $P = 0.121$; Dose effect of YS when Cr included in diet, linear, $P = 0.197$, quadratic, $P = 0.041$.
[6] Dietary phase 1 to 3 fed for d-0 to 69 for both barns.
[7] Dietary phase 4 and 5 fed for d-69 to 103 and d-69 to 113, barn 1, barn 2, respectively.
[8] Dose effect of YS when Cr not included in diet, linear, $P = 0.193$, quadratic, $P = 0.109$; Dose effect of YS when Cr included in diet, linear, $P = 0.209$, quadratic, $P = 0.026$.
[9] Dose effect of YS when Cr not included in diet, linear, $P = 0.700$, quadratic, $P = 0.414$; Dose effect of YS when Cr included in diet, linear, $P = 0.031$, quadratic, $P = 0.667$.
[10] The largest 3 pigs were marketed from each pen on d-95 for barn 1, and d-98 for barn 2. All remaining pigs were marketed from each pen on d-103 and d-113, respectively. Carcass characteristics other than yield were adjusted to a common HCW by inclusion of HCW as a covariate in the statistical model.
[11] Yield was calculated by dividing HCW by average pen live weight collected at the research barn prior to transport to processing facility.

Chapter 6 - Effect of dietary medium chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage[6]

ABSTRACT

An experiment was conducted to evaluate the effect of dietary medium chain fatty acid (MCFA) addition on nursery pig growth performance, fecal microbial composition, and mitigation of porcine epidemic diarrhea virus (PEDV) following storage. A total of 360 pigs (DNA 400 × 200, Columbus, NE; initially 6.7 ± 0.07 kg) were randomized to pens (5 pigs per pen) on the day of weaning (approximately 20 d of age), allowed a 6-d acclimation, blocked by body weight (BW), and randomized to dietary treatment (9 pens per treatment). All MCFA (Sigma Aldrich, St. Louis, MO) were guaranteed ≥ 98% purity, including hexanoic (C6:0), octanoic (C8:0), and decanoic (C10:0) acids. Treatment diets were formulated in two phases (7 to 11 and 11 to 23 kg BW) and formulated to meet or exceed NRC requirement estimates. Treatments (n = 8) were a dose response including 0, 0.25, 0.5, 1.0, and 1.5% added MCFA blend (1:1:1 ratio C6:0, C8:0, and C10:0), as well as treatments with individual additions of 0.5% C6:0, C8:0, or C10:0. Fecal samples were collected from pigs fed control and 1.5% MCFA blend diets on d 0 and d 14 and

[6] This work has been published in *Journal of Animal Science*: J. T. Gebhardt, K. A. Thomson, J. C. Woodworth, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, C. K. Jones, R. A. Cochrane, M. C. Niederwerder, S. Fernando, W. Abbas, and T. E. Burkey. 2019. Effect of dietary medium chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. J. Anim. Sci. (In-press). doi:10.1093/jas/skz358.

analyzed using 16s rDNA sequencing. Following feed manufacture, feed was stored in bags at barn temperature and humidity for 40 d before laboratory inoculation with PEDV. Subsamples of retained feed were inoculated with PEDV to achieve a titer of 10^4 $TCID_{50}$/g and separate sample bottles were analyzed on 0 and 3 days post-inoculation (dpi). Overall, average daily gain (ADG) and average daily feed intake (ADFI) were increased (linear, $P \leq 0.010$) and feed efficiency (G:F) improved (linear, $P = 0.004$) with increasing MCFA blend. Pigs fed 0.5% C8:0 had greater ($P = 0.038$) ADG compared to pigs fed the control diet, and G:F was improved ($P \leq 0.024$) when pigs were fed 0.5% C6:0, 0.5% C8:0, or 0.5% C10:0 compared to control. An inclusion level × day interaction was observed (quadratic, $P = 0.023$), where PEDV Ct values increased (quadratic, $P = 0.001$) on 0 dpi with increasing levels of MCFA blend inclusion and also increased on 3 dpi (linear, $P < 0.001$). Fecal microbial diversity and composition were similar between control and 1.5% MCFA blend. In summary, the use of MCFA in nursery pig diets improves growth performance, provides residual mitigation activity against PEDV, and does not significantly alter fecal microbial composition.

Keywords: medium chain fatty acid, microbiome, nursery, PEDV, pig

INTRODUCTION

Medium chain fatty acids (MCFA) have been shown to mitigate the risk of porcine epidemic diarrhea virus (PEDV) transmission via feed and ingredients (Cochrane et al., 2016; 2017; Dee et al., 2016). In addition to feed pathogen mitigation, research has evaluated the impact of MCFA on growth performance (Zentek et al., 2011). Various mechanisms by which MCFA may influence growth performance have been described, including its use as a readily available energy substrate, modifier of gastrointestinal morphology, and antibacterial compound (Hanczakowska et al., 2017). However, uncertainty still exists largely due to variability in the

MCFA content of various sources available to the industry, and what inclusion level is necessary to optimize performance. Thus, additional research is necessary to further characterize the effects of dietary MCFAs on growth performance, shifts in fecal microbiome composition, and the value of feed-borne pathogen risk reduction. One of the main advantages of adding MCFA to feed is residual mitigation characteristics following feed manufacture and storage (Gebhardt et al., 2019). The ability for MCFA to retain antiviral properties post-manufacturing provides the opportunity to mitigate pathogen contamination during transport or at the farm. However, to date, no research has been conducted quantifying residual mitigation properties of MCFA against PEDV beyond one-day post feed manufacturing. Therefore, the objective of this experiment is to 1) determine the impact of hexanoic (C6:0), octanoic (C8:0), and decanoic (C10:0) acid supplementation in nursery pig diets on growth performance and fecal microbial composition, and 2) PEDV mitigation activity in complete feed following several weeks of feed storage.

MATERIALS AND METHODS

Animals and Diets

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State Segregated Early Weaning Facility in Manhattan, KS. Following arrival on the day of weaning (approximately 20 d of age), pigs were randomized to pens and allowed a 6-d acclimation period during which they were fed a commercial starter pellet containing no feed-grade antimicrobials. Following acclimation, 360 pigs (DNA 400 × 200; Columbus, NE, initially 6.7 ± 0.07 kg BW) were blocked by BW and randomized to dietary treatment. Treatment diets were formulated and manufactured in two dietary phases (phase 1 = 7 to 11 kg BW; phase 2 = 11 to 23 kg BW). Diets were formulated to meet or exceed NRC (2012) requirement estimates for nutrients and energy.

Phase 1 diets included 1,910 mg/kg added zinc (1,800 mg/kg from zinc oxide and 110 mg/kg from zinc sulfate) and 17 mg/kg added copper from copper sulfate. Phase 2 diets included 110 mg/kg added zinc from zinc sulfate and 17 mg/kg added copper from copper sulfate. Medium chain fatty acids (Sigma Aldrich, St. Louis, MO, USA) included C6:0, C8:0, and C10:0 and were guaranteed ≥ 98% purity. Treatments (n = 8) were constructed such that a dose response was created including 0, 0.25, 0.5, 1.0, and 1.5% added MCFA blend (1:1:1 ratio C6:0, C8:0, and C10:0), as well as treatments with 0.5% of either C6:0, C8:0, or C10:0. Each pen had tri-bar floors and contained a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Pens (1.22 × 1.22 m) each contained 5 pigs and allowed approximately 0.298 m^2/pig. Pig weights and feed disappearance were measured on d 0, 7, 14, 21, 28, and 35 to determine average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). Fecal consistency was evaluated on d 8, 11, 14, 21, 28, and 35 by three, trained, independent reviewers per day. Scoring was performed on a 5-point scale with 1 = hard, pellet-like feces; 2 = firm, formed stool; 3 = soft, moist stool that retains shape; 4 = unformed stool; and 5 = watery liquid stool. When consensus was not achieved within reviewers to provide single fecal score, the concordant score was considered the definite score to be included in the statistical model as described by Menegat et al. (2019).

Chemical Analysis

Complete diet samples were collected following feed manufacture using a feed probe from every fifth bag, subsampled, and submitted (Ward Laboratories, Inc., Kearney, NE, USA) for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), crude fiber (AOAC 978.10, 2006), Ca (AOAC 965.14/985.01, 2006), P (AOAC 965.17/985.01, 2006), and ether extract (AOAC 920.39 A, 2006). In addition, free fatty acid (C6:0, C8:0, C10:0; AOCS, 2017) concentrations were

determined at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO, USA).

Feed Sample Collection and PEDV Inoculation

Following manufacture, all treatment diets were stored in 22.7 kg paper bags at barn temperature and humidity at the Kansas State University Segregated Early Wean facility for 40 d (June to July 2017). Meteorological data was collected using Weather Underground (wunderground.com) for the closest sampling station (Manhattan, Kansas Regional Airport; 11.9 km) for the dates of diet storage (average ± standard deviation; average daily average temperature = 25.8 ± 3.52 °C, average daily high temperature = 33.1 ± 3.67 °C, average daily low temperature = 18.3 ± 3.99 °C, average daily maximum humidity = 90 ± 8.8%, average daily minimum humidity = 41 ± 9.4%). Following storage, samples were collected from multiple bags per treatment and subsampled using a riffle-splitter. Six, 22.5 g samples of each treatment diet were placed in separate 250 mL high density polyethylene (HDPE) bottles (Thermo Fisher Scientific, Waltham, MA, USA) to be inoculated with PEDV and analyzed on two sampling days post-inoculation (0 and 3 days post-inoculation; dpi), with 3 replications of each sampling day and treatment combination. In addition, 22.5 g of feed without MCFA was added to three separate bottles as control samples which were not inoculated with PEDV and were analyzed along with the 0 dpi inoculated bottles.

Inoculation was carried out at the Kansas State University Veterinary Diagnostic Laboratory. The PEDV inoculum was cell culture derived (USA/IN/2013/19338, passage 9) and had an initial concentration of 10^5 $TCID_{50}$/mL. Inoculation occurred by pipetting 2.5 mL of inoculum into each bottle containing 22.5 g feed matrix, resulting in an inoculated feed matrix with a viral concentration of 10^4 $TCID_{50}$/g of feed matrix. Following the addition of the viral

inoculum to each bottle, the bottles were lightly shaken in a circular pattern for approximately 5 s to prevent material from sticking to the side of the bottles. After which, each bottle was vigorously hand shaken for approximately 10 s to mix the virus evenly throughout the feed. The three negative control bottles had 2.5 mL of PBS added to each bottle as a sham inoculation following similar procedures to the viral inoculation. Samples were stored at room temperature until analysis performed on appropriate dpi.

Real-Time PCR Analysis

Separate bottles were analyzed on 0 and 3 dpi. On the day of inoculation (0 dpi), processing of the inoculated 0 dpi bottles occurred within three hours of inoculation. On each day of analysis, 100 mL phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY, USA) was added to each bottle predetermined for analysis on that day. Bottles were shaken for approximately 10 s, at which point they could settle overnight at 4ºC. The following day, supernatant was pulled and aliquoted for further analysis. A total of 2 aliquots from each sample bottle were collected and stored at -20ºC until the conclusion of the trial, at which point qRT-PCR analysis was performed on one aliquot per sample bottle. The qRT-PCR was conducted at the Kansas State University Veterinary Diagnostic Laboratory as previously described (Gebhardt et al., 2019). Values are reported as cycle threshold (Ct), where an increase in Ct value indicates less genetic material present in the analyzed sample.

Fecal Microbial population analysis

Fecal samples were collected from one pig per pen within control and 1.5% MCFA blend (1:1:1 ratio C6:0, C8:0, and C10:0) treatments (9 pens/treatment) on d 0 and 14 of the study period. The same pig within each pen was sampled on both dates. Sterile cotton tipped applicators (Puritan Medical Products, Guilford, ME, USA) were inserted into the rectum to

stimulate defecation. One applicator was used per pig per time point. Fecal samples were collected into clean, single use zipper storage bags and were then transferred into 3 mL cryovials and stored at -80°C until shipment to the University of Nebraska, Lincoln for DNA extraction and bacterial community analysis.

DNA was extracted using the manufacturer's protocol for Mag-Bind Soil DNA 96 Kit (Omega Bio-tek, Inc., Norcross, GA, USA) with the following modifications: precipitation of nucleic acids was done by using sodium acetate, isopropanol and ethyl alcohol. Each sample tube received 0.1× volumes of 10 mM sodium acetate, which were vortexed and later incubated on ice for 5 min. Subsequently, 1ml of ice-cold isopropanol was added and samples were incubated at -80°C overnight to precipitate the DNA. The following day, samples were centrifuged at 4°C for 15 min at 16,000 × g. The supernatants of the resulting samples were discarded and the nucleic acid pellet was washed with 0.5ml of ice-cold 70% ethyl alcohol. The samples were centrifuged for 2 min at 13,000 x g, the residual supernatant was discarded, and the nucleic acid pellet was air dried for 3 min. The nucleic acid pellet was dissolved in a 0.45ml of Tris [tris(hydroxymethyl)aminomethane; 10mM, pH 8] and incubated for 1 hr at 4°C. For further purification of dissolved nucleic acids, the KingFisher (ThermoFisher Scientific, Waltham, MA, USA) robot was used with reagents from the Mag-Bind Soil DNA 96 Kit. The resulting DNA was used for the tag-sequencing of the V4 region of 16S rDNA using the universal bacterial primers described previously (Kozich et al., 2013). A 20 µL PCR reaction contained 1× TerraTM PCR Direct Polymerase Mix, 0.5 µL Terra polymerase, 20 mM of each primer, and 20-50 ng of DNA. The cycling conditions for PCR were the same as previously described (Paz et al., 2016). The PCR product size was confirmed by agarose gel electrophoresis. Normalization of the amplified PCR products were performed with Just-a-Plate 96 PCR Purification & Normalization

kit (Charm Biotech, San Diego, CA, USA) according to the manufacturer's protocol. Following normalization, 10 μL from each sample were pooled and concentrated using Nucleospin Gel & PCR Cleanup kit (MACHEREY-NAGEL Gmbh & Co. KG, Duren, Germany) and was eluted using 20 μL of elution buffer. This pooled and purified sample was analyzed in an Agilent 2100 bioanalyzer (Agilent Scientific Instruments, Santa Clara, CA, USA) using Agilent High Sensitivity DNA Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) to ensure the quality and quantity of the targeted V4 region of 16S rDNA. The concentration of the DNA library was determined using the DeNovix QFX Fluorometer (DeNovix Inc., Wilmington, DE, USA) and using DeNovix dsDNA Fluorescence Quantification Assay (DeNovix Inc., Wilmington, DE, USA). The resulting 16S rDNA libraries were sequenced using the Illumina MiSeq platform utilizing the 2 × 250 paired end sequencing strategy using a MiSeq Reagent Kit V3 (Illumina Inc., San Diego, CA, USA).

Data processing was performed on a custom pipeline utilizing several publicly available software tools. The paired-end reads were assembled into contiguous sets of overlapping clones (contigs) after quality filtering using MOTHUR v.1.38.1 as previously described (Schloss et al., 2009). Operational taxonomic units (OTUs) were generated from the quality filtered sequences using the UPARSE pipeline (USEARCH v7.0.1090) at a threshold of 97% identity as previously described (Edgar, 2013). Chimeric sequences were removed using the ChimeraSlayer gold.fa as the reference database using UCHIME (Edgar et al., 2011). OTUs were aligned against the v128 (SILVA) database and mismatched sequences were discarded. A phylogenetic tree was generated using high quality aligned sequences within MOTHUR v.1.38.1 using the Clearcut algorithm as previously described (Sheneman et al., 2006). Taxonomies to the identified OTUs were assigned using QIIME v.1.9.1 pipeline (Caporaso et al., 2010) with the Greengenes reference database

(gg_13_5_otus). OTUs representing Archaea and Cyanobacteria were removed as Cyanobacterial reads may be a result of contamination of plant chloroplast (Giovannoni et al., 1988) and the archaea sequences may be biased as the primers used are not designed to universally amplify all archaea. Alpha diversity matrices (Chao1 and Observed OTUs) were calculated using the QIIME v.1.9.1 pipeline. The rarefaction of the OTU table was performed using QIIME v.1.9.1 with the lowest number of reads (Edgar et al., 2011). For the experiment, 29,663 was used as the lowest depth.

Statistical Analysis

Growth data were analyzed as a randomized complete block design with pen as the experimental unit. Weight block was included in the statistical model as a random effect. Additionally, fecal score and PEDV inoculation data were evaluated as a repeated measures over time. All comparisons incorporated Bonferroni multiple comparison adjustments within preplanned pairwise contrasts comparing individual 0.5% MCFA supplemented diets to 0.5% 1:1:1 MCFA treatment and control. Within outcomes, linear and quadratic effects of increasing MCFA blend were evaluated. Fecal consistency was evaluated assuming a multinomial distribution and considering the frequency distribution of pens within each fecal score category using a single determinate fecal score per pen per day of evaluation. An unstructured or first order ante-dependence covariance structure were evaluated for fecal consistency scores due the unequal nature of the evaluation days. The first order ante-dependence was selected for use based on an improved Bayesian Information Criterion relative to the unstructured covariance matrix. Statistical analysis was performed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). The FREQ procedure of SAS version 9.4 (SAS Institute,

Inc., Cary, NC, USA) was used to calculate frequency distribution of fecal scores within each fecal score category.

Fecal microbial population relative abundance was analyzed using a linear mixed model with individual pig as the experimental unit representing the single sample collected from each pen on a given sampling day. Relative abundance was considered the proportion of total reads for a specific sample classified into the designated microbial phyla or family and was analyzed assuming a binomial response distribution with the numerator being the number of reads within a sample for a specific phyla or family and the denominator being the total number of reads for the sample. Day of analysis, treatment, and the associated interaction were included in the statistical model as fixed effects. A repeated measure statement was used to account for repeated sampling of the same set of pigs on both d 0 and d 14. Means separation was performed using the DIFF option to perform pairwise comparisons. The Kruskal-Wallis test was performed on the Chao1 and observed OTU's to assess the bacterial richness difference among control and 1.5% MCFA blend treatments on d 14 using the NPAR1WAY procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). All results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Chemical analysis of diets (Table 2) reflected formulated values. The analyzed MCFA content closely matched formulated values, indicating manufacturing processes were effective in minimizing MCFA crossover between treatment diets. Ether extract decreased as MCFA was increased and soybean oil inclusion was decreased indicating that ether extract is not an efficient method to detect C6:0, C8:0, and C10:0 under these conditions.

Growth Performance

During dietary phase 1, pigs fed increasing MCFA blend had increased (Tables 3 and 4; linear, $P \leq 0.003$) ADG and ADFI, as well as improved G:F (quadratic, $P = 0.021$). Pigs fed 0.5% C8:0 had greater ($P = 0.007$) ADG than pigs fed the control diet. Pigs fed 0.5% C6:0, 0.5% C8:0, or 0.5% C10:0 had improved G:F ($P \leq 0.044$) compared to control fed pigs. During phase 2, ADG increased (linear, $P = 0.007$) and ADFI marginally increased (linear, $P = 0.052$) with increasing MCFA blend. Overall, ADG, ADFI, and G:F increased (linear, $P \leq 0.010$) with increasing MCFA blend. Pigs fed 0.5% C8:0 had greater ($P = 0.038$) ADG compared to pigs fed the control diets, and G:F was improved ($P \leq 0.024$) when pigs were fed 0.5% C6:0, 0.5% C8:0, or 0.5% C10:0 compared to control. No evidence of a difference in BW, ADG, ADFI, or G:F was observed for dietary phase 1, dietary phase 2, or overall between individual MCFA at 0.5% inclusion compared to 0.5% inclusion of MCFA blend ($P \geq 0.467$).

No evidence for a dietary treatment × day interaction was observed ($P = 0.663$; Figure 1) for fecal consistency. At all points of evaluation, the majority of determinant fecal scores were characterized as firm formed stool, soft, moist stool that retains shape, or unformed stool. As expected, a significant day effect was observed ($P < 0.0001$). Over the course of the study, fecal consistency transitioned to a firmer pattern, with a decrease in frequency distribution of pens with soft, moist feces that retains shape and an increase of pens with firm, formed stool. There was no evidence that treatment had an effect on fecal consistency ($P = 0.211$).

Mitigation characteristics

A MCFA blend inclusion level × day interaction was observed (quadratic, $P = 0.023$; Table 3). This was the result of PEDV Ct values increasing in a quadric manner ($P = 0.001$) as inclusion rates increased on 0 dpi while increasing in a linear manner on 3 dpi (linear, $P <$

0.001). On 0 dpi, the addition of 0.5% C6:0, 0.5% C8:0, or 0.5% C10:0 resulted in significantly greater Ct values compared to control ($P \leq 0.003$). Inclusion of 0.5% C6:0 or 0.5% C10:0 resulted in lower Ct values ($P \leq 0.047$) on 0 dpi compared to 0.5% MCFA blend. Insufficient evidence was available on 0 dpi to demonstrate a difference in Ct value between 0.5% C8:0 and 0.5% MCFA blend treatments ($P = 0.394$). On 3 dpi, the addition of 0.5% C6:0 or 0.5% C10:0 resulted in greater Ct values compared to control ($P \leq 0.004$), with marginally significant evidence that the 0.5% C8:0 treatment Ct value was greater than control ($P = 0.061$). Sufficient evidence was lacking on 3 dpi to demonstrate a difference in Ct value between 0.5% MCFA blend treatment and treatments consisting of individual MCFA at 0.5% inclusion ($P = 1.000$).

Fecal microbial population analysis

The majority of bacterial sequences were classified in two phyla on d 0 and 14, and consisted of Firmicutes and Bacteroidetes (Figure 2). A total of 6 phyla were found at ≥ 1% relative abundance for at least one of the treatment × day analysis combinations. Phyla and families with low (< 1%) relative abundance were excluded from the data analysis. Overall, fecal microbial populations were similar between pigs fed 1.5% MCFA compared to control pigs. A marginally significant treatment × day interaction was observed in the Proteobacteria phylum ($P = 0.057$), where relative abundance was consistent over time in pigs fed the control diet ($P = 0.668$; 1.5 ± 0.52, 2.1 ± 0.60%; d 0, d 14, respectively), whereas a decrease over time was observed in pigs fed the 1.5% MCFA blend diet ($P = 0.023$; 1.2 ± 0.46, 0.1 ± 0.16%; d 0, d 14, respectively). There was no evidence of a difference in the effect of MCFA addition over time for the relative abundance of the remaining phyla (treatment × day, $P \geq 0.231$).

The main effect of day indicated a significant increase over time in the Tenericutes phylum ($P = 0.016$). A marginally significant increase over time was observed in the Firmicutes

($P = 0.096$) and Actinobacteria ($P = 0.088$) phyla. A significant decrease over time was observed for the Proteobacteria phylum ($P = 0.026$) and Spirochaetes phylum ($P = 0.022$). A Firmicutes:Bacteroidetes ratio was calculated, and there was no evidence of a treatment × day interaction ($P = 0.338$) or day effect ($P = 0.211$).

A total of 22 families were found at ≥ 1% relative abundance for at least one of the treatment × day of analysis combinations. The families with the greatest relative abundance were *Prevotellaceae*, *Ruminococcaceae*, and S24-7 which each had ≥ 10% relative abundance for at least one of the treatment × day analysis combinations. There was no evidence of a difference in the effect of MCFA addition over time for the relative abundance for any family (treatment × day, $P \geq 0.138$). A reduction over time was observed for *Ruminococcaceae* ($P = 0.032$), *Lachnospiraceae* ($P = 0.006$), *Christensenellaceae* ($P = 0.013$), *Spirochaetaceae* ($P = 0.033$), *Bacteroidaceae* ($P = 0.014$), and *Succinivibrionaceae* ($P = 0.039$).

An increase in relative abundance over time was observed for the unclassified Clostridiales ($P = 0.014$), *Clostridiaceae* ($P < 0.0001$), unclassified RF39 ($P = 0.016$), and Clostridiales; other ($P = 0.001$) families. At d 14, no evidence of a difference in alpha diversity was observed for Chao1 or observed OTU's between control and 1.5% MCFA blend treatments ($P > 0.10$) was observed.

DISCUSSION

Feedstuffs delivered to swine farms are a potential source of disease transmission (Pasick et al., 2014; Bowman et al., 2015, Aubry et al., 2017). Mitigation of transmission risk through feed has clear and definable economic benefits. The use of MCFA compounds to mitigate the risk of PEDV transmission via feed or feed ingredients has significant scientific promise (Cochrane et al., 2016; 2017; Dee et al., 2016). In addition to feed pathogen mitigation, MCFAs

have been evaluated as compounds to improve growth performance as reviewed by Zentek et al. (2011) and Hanczakowska (2017). However, uncertainty still exists due to variability in the MCFA content of various sources available to the industry, and what inclusion level is necessary to optimize performance. Potential mechanisms by which MCFA may influence growth performance are diverse, and include use as a readily available energy source, modifier of gastrointestinal morphology, and modifier of gastrointestinal microbial populations through anti-bacterial properties (Zentek et al., 2011; Hanczakowska et al., 2017).

Medium chain fatty acids are unbranched, saturated monocarboxylic acids containing between 6 and 12 carbon atoms (Zentek et al., 2011). In nature, these compounds are often in triglyceride form (medium chain triglyceride; MCT) with greatest concentrations in coconut and palm kernel oil (Zentek et al., 2011). To date, MCFA studies conducted by our research group regarding mitigation of feed-borne pathogens have utilized the free fatty acid forms. Depending on the chemical structure (MCFA vs. MCT), digestion, absorption, and utilization differ. Due to the water soluble nature of MCFA, they are rapidly absorbed within the small intestine and can be used by enterocytes as an energy substrate, or travel to the liver to undergo β-oxidation in hepatocytes (Zentek et al., 2011). In order to accurately assess the value of MCFA utilization for enhancement of growth performance in combination with previous research evaluating efficacy as a feed-borne pathogen mitigant, the current experiment utilized the free fatty acid form.

The effects of MCFA inclusion in swine diets on growth performance have recently been reviewed by Hanczakowska (2017) with multiple studies demonstrating positive effects on gain and feed efficiency. In the current experiment, addition of MCFA resulted in an improvement in growth performance compared to control, particularly C6:0 and C8:0 fatty acids for ADG and C6:0, C8:0, or C10:0 for G:F. Hanczakowska et al. (2011b) observed an increase in ADG when

0.2% C8:0, 0.2% C10:0, and a combination of 0.1% C8:0 and 0.1% C10:0 were included in swine diets beginning at 7 days of age compared to control, and feed efficiency was also improved when pigs were fed 0.2% C8:0 compared to control. The levels of MCFA used in the current experiment were significantly higher than Hanczakowska et al. (2011b); however, similar improvements in growth performance were observed. Hanczakowska et al. (2013) observed an increase in ADG when pigs were fed C8:0, C10:0, or combination of C8:0 and C10:0 in diets containing propionic and formic acids compared to control fed pigs; however, ADG did not significantly differ comparing pigs fed MCFA, propionic, and formic acids to propionic and formic acids alone. Mohana and Kim (2014) did not observe any improvement in growth performance when pigs were supplemented 0.2% MCFA product, which contained 58% C8:0 and C10:0, compared to control-fed pigs. Largely, published literature has shown that MCFA supplementation improves growth performance. Variability in presence and magnitude of effect is likely driven by differences in MCFA origin and dose, further reinforcing the need for greater understanding of optimum dose.

Several studies have shown evidence that inclusion of MCFA in pig diets of varying ages can improve gastrointestinal morphology through increased villus length and associated increased villus height:crypt depth ratio (Dierick et al., 2003; Hanczakowska et al., 2011a; 2011b; Chwen et al., 2013). Contrary to these findings, other studies did not find evidence of a difference in villus height or crypt depth when MCFA were fed to young pigs (Hanczakowska et al., 2016; Ferrara et al., 2017). Increased surface area for nutrient digestion and absorption is generally believed to be beneficial, and Han et al. (2011) observed an increase in nutrient digestibility including energy, crude protein, calcium, phosphorous, and amino acids when pigs were fed a micro-encapsulated eucalyptus-medium chain fatty acid product. The current study

did not evaluate morphologic changes in the gastrointestinal tract or nutrient digestibility; however, feed efficiency was improved with MCFA supplementation. Further evaluation of gastrointestinal morphology using the free fatty acid form used in the current experiment at the dose range utilized would be necessary to draw any associations between the observed improvements in growth and feed efficiency and changes to morphology attributed to MCFA.

Pure MCFA commonly have a foul odor and have been shown to reduce feed intake as described by Hanczakowska (2017). Cera et al. (1989) observed a reduction in intake when C8:0 and C10:0 fatty acid mixture was fed to weaned pigs; however, it was not known whether the reduction was due to odor characteristics or nutritional adjustment to increased energy availability. To the contrary, multiple studies have shown no evidence of reduced feed intake when MCFA are fed (Hanczakowska et al., 2011a; Mohana and Kim, 2014; Hanczakowska et al., 2016) which is consistent with our data. The observed differences among previous studies may be attributed to differences in MCFA inclusion level as well as origin of the MCFA. The MCFA used in the current experiment created a strong odor both in pure form and when mixed into feed. The strong odor of the MCFA used in the current experiment did not reduce feed intake, but rather led to a linear increase with increasing MCFA blend inclusion.

It has been observed that weaned pigs have firmer stool when fed pharmacological copper and zinc doses shortly after weaning, which also correlates with improved growth (Hill et al., 2000; Shelton et al., 2011). It is also accepted that clinical enteric disease can create diarrhea and can significantly reduce growth performance. In the current experiment, no known significant disease pressure was present, and firmer stools were observed over time. Contributing factors for the observed impact on stool firmness could include changes in gastrointestinal microbial populations unable to be identified in fecal analysis or modulation of gastrointestinal

water and ion balance over time. A greater understanding of the underlying physiology is necessary prior to inferring causation for changes in fecal consistency.

Generally, MCFA are considered to have inhibitory effects on growth and composition of bacterial populations (Zentek et al., 2011). Although control of pathogenic bacterial populations is beneficial, commensal bacterial populations play a significant role in nutrient metabolism and immune system development (Mach et al., 2015). The mechanism by which MCFA exert their antibacterial properties is not fully understood; however, it is likely due to amphiphilic structure that allows for disruption of cellular membranes, leakage of intracellular materials, and subsequent cellular death (Zhou et al., 2019). Zentek et al. (2011) and Hanczakowska (2017) summarized both *in vivo* and *in vitro* experiments evaluating the effects of MCFA on microbial populations, and concluded the antimicrobial properties of C8:0 and C10:0 are most consistent against Gram-positive bacteria, with limited activity against Gram-negative species. Recent *in vitro* experiments have shown that using a 1:1:1 ratio of C6:0:C8:0:C10:0 MCFA has the lowest minimum inhibitory concentration (MIC) against generic *Escherichia coli*, and C6:0 has the lowest MIC for enterotoxigenic *Escherichia coli* (Swanson et al., 2018). Additionally, Sylvester et al. (2018) observed that C6:0 and C8:0 had lower MIC for *Salmonella* Typhimurium compared to C10:0 or 1:1:1 ratio of C6:0:C6:0:C10:0 MCFA. Shilling et al. (2013) observed that C12 was more inhibitory towards growth of *Clostridium difficile* than C6:0 or C8:0 MCFA. *Escherichia coli* and *Salmonella* are both Gram-negative bacterial species, whereas *Clostridium difficile* is a Gram-positive bacterium. Thus, the efficacy of shorter length MCFA compounds are generally more effective at inhibiting growth of Gram-negative species whereas Gram-positive species are more effectively controlled with longer length MCFA. Previous literature would suggest that while MCFA have been shown to have antibacterial properties, the effectiveness

differs among combination of MCFA and bacterial species. In an effort to further characterize the impact of MCFA supplementation on the fecal microbial populations, the current experiment evaluated the fecal microbial populations on d 0 and d 14 using 16s rDNA sequencing.

Consistent with previous literature evaluating microbial populations at multiple locations within the gastrointestinal tract (Li et al., 2018; Pollack et al., 2018; Yang et al., 2017), the largest proportion of microbes in the current study were characterized as part of the Firmicutes and Bacteroidetes phyla. At the family level, Li et al. (2018) found *Prevotellaceae* to be most abundant family in the colon of piglets around the time of weaning, which is consistent with the current study. The microbial composition within different locations in the gastrointestinal tract substantially differs (Zhao et al., 2015), and fecal characterization likely only represents a subset of total gastrointestinal microbial populations (Quan et al., 2018). The current study only evaluated fecal microbial populations; thus, differences in microbial populations in the small or large intestine would have gone undetected using current methodologies.

Addition of MCFA resulted in minor alterations in fecal bacterial populations. A marginally significant interaction characterized by a reduction in Proteobacteria over time in pigs fed MCFA compared to no change in controls was the only change associated with MCFA supplementation. Proteobacteria is a diverse phyla of Gram-negative bacteria including the genera *Escherichia* and *Salmonella* (Gupta, 2000). In humans, high levels of Proteobacteria are associated with unstable microbial communities, commonly known as dysbiosis (Shin et al., 2015). In the current study, the marginally significant reduction in Proteobacteria over time caused by MCFA may have been a contributing factor to improved growth performance. However, no evidence for differences on a family level were associated with MCFA supplementation over time. It is possible that significant changes in community structure could

take longer than 14 days and thus would not be identified within the current experiment. Additionally, further characterization of the microbiome, using metagenomic sequencing or the evaluation of non-bacterial microbial composition, may reveal a more significant impact of MCFA on the gut microbial populations. Nonetheless, the current study suggests that the positive impact associated with feeding MCFA on growth may not be directly related to substantial shifts in fecal microbial populations.

With recent advances in high throughput quantification of microbial populations, research focusing on the impact of gastrointestinal microbial populations on feed efficiency is present (McCormack et al., 2017; Maltecca et al., 2019; McCormack et al., 2019). The results of these studies identify multiple taxonomic classifications that are observed to be associated with feed efficiency and growth performance outcomes. In one such investigation, pigs with greater feed efficiency than their counterparts were observed to have greater abundance of the *Lachnospiraceae* and *Prevotellaceae* families along with the *Escherichia-Shigella* and *Streptococcus* genera (Quan et al., 2019). Additionally, advances in knowledge of the role specific organisms play in metabolism of starch, fructans, and lactose has improved which may lead to improved theories regarding efficiency of nutrient utilization and growth performance (Wang et al., 2019). Although the overall microbial community remained largely unaffected by the addition of 1.5% MCFA in the diet, time had a significant influence on relative microbial abundance. It is understood that as young pigs age and transition from a milk-based diet to dry feed, their digestive enzyme profile and gastrointestinal microbial profile adjust (Hu et al., 2016, Chen et al., 2017; Guevarra et al., 2019). Multiple changes in relative abundance were observed over time, indicating that the experimental methods were sensitive to changes in microbial

populations and provides further understanding of the changes in microbial populations post-weaning.

The use of MCFA to mitigate PEDV in swine diets has been evaluated by Cochrane et al. (2016) and reported to reduce quantifiable genetic material and infectivity characteristics. To date, no research has been conducted quantifying the residual mitigation properties of MCFA beyond one-day post feed manufacturing. In contrast, the use of 37% formaldehyde has been approved to be added to livestock feed or feed ingredients to maintain *Salmonella*-negative status for up to 21 days (FDA, 2003). We believe this is the first study to demonstrate long term mitigation potential for MCFA against PEDV. In the current experiment, feed treated with MCFA retained mitigation activity as quantified by an increase in Ct value when inoculated with PEDV following a storage period of 40 days. Thus, the current study provides a baseline understanding of the timeline associated with the residual duration of activity that treatment of feed with MCFA provides. An important limitation of the current study was lack of evaluation of infectivity characteristics post-inoculation using methods such as virus isolation or bioassay. The use of swine bioassay models to evaluate the infectivity of feed samples inoculated with PEDV have been shown to be a robust experimental model (Schumacher et al., 2016). The current experiment was preliminary in nature and set out to determine residual mitigation activity as quantified using qRT-PCR as a baseline for further investigation. Additional investigation is required to fully understand the level of risk reduction for post-processing contamination that can be expected when using MCFA in swine feed.

In summary, treating swine nursery feed with MCFA improved growth performance, resulted in sustained mitigation properties when inoculated with PEDV following a period of feed storage, and does not significantly alter fecal microbial populations. The use of such

compounds may provide benefit to swine producers from both a reduction in disease transmission risk as well as improved growth performance. Further investigation into the role of added dietary MCFA including sources that would be practical and cost effective on growth performance, gastrointestinal bacterial flora, and residual mitigation properties against feed-borne pathogens is warranted.

LITERATURE CITED

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Table 6.1. Diet composition (as-fed basis)

	Weight range, kg	
Item	7 to 11	11 to 23
Ingredient, %		
Corn	54.92	62.55
Soybean meal (46.5% CP)	26.38	31.60
Whey powder	10.00	- - -
Enzymatically-treated soybean meal[2]	2.50	- - -
Soybean oil	1.50	1.50
Calcium carbonate	0.95	1.00
Monocalcium phosphate (21% P)	1.30	1.15
Salt	0.60	0.60
L-Lysine HCl	0.50	0.51
DL-Methionine	0.24	0.23
L-Threonine	0.21	0.21
L-Tryptophan	0.05	0.06
L-Valine	0.15	0.14
Trace mineral[3]	0.15	0.15
Vitamin premix[4]	0.25	0.25
Phytase[5]	0.07	0.07
Zinc oxide	0.25	- - -
Hexanoic acid, C6:0[6]	+/-	+/-
Octanoic acid, C8:0[6]	+/-	+/-
Decanoic acid, C10:0[6]	+/-	+/-
Total	100	100
Calculated analysis[7]		
Standardized ileal digestible (SID) amino acids, %		
Lysine	1.35	1.35
Isoleucine:lysine	56	55
Leucine:lysine	111	113
Methionine:lysine	37.4	37.3
Methionine & Cystine:lysine	58.2	58.1
Threonine:lysine	63.0	62.0
Tryptophan:lysine	20.1	20.3
Valine:lysine	70.3	70.1
Total lysine, %	1.48	1.49
ME, kcal/kg	3,349	3,347
NE, kcal/kg	2,502	2,485
SID Lys:ME, g/Mcal	4.03	4.03
SID Lys:NE, g/Mcal	5.40	5.43
CP, %	20.6	21.1
Ca, %	0.75	0.70
P, %	0.68	0.63
STTD P, %	0.57	0.50

[1] Treatment diets were fed to 360 pigs [DNA 400 × 200 (Columbus, NE); initial BW = 6.7 ± 0.07 kg] for 35-d in a 2-phase feeding program with 5 pigs per pen and 9 pens per treatment.

[2] HP300 (Hamlet Protein, Findlay, OH).

[3] Premix provided per kg of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 26.4 g Mn from manganese oxide; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[4] Premix provided per kg of premix: 4,409,249 IU vitamin A; 551,156 IU vitamin D3; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 19,842 mg niacin; 11,023 mg pantothenic acid; and 3,307 mg riboflavin.

[5] HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided an estimated release of 0.12% STTD P.

[6] Sigma Aldrich (St. Louis, MO), guaranteed ≥ 98% purity added at the expense of soybean oil in appropriate treatment diets.

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

Table 6.2. Analyzed diet composition (as-fed basis)[1]

	Added MCFA, %[2]							
		C6:0:C8:0:C10:0[3]				C6:0	C8:0	C10:0
Analyzed composition, %[4]	0	0.25	0.5	1.0	1.5	0.5	0.5	0.5
7 to 11 kg								
DM	89.15	89.02	89.02	88.91	88.58	89.66	89.22	89.10
CP	20.90	20.70	21.20	20.90	20.45	20.85	20.50	19.80
ADF	3.90	3.35	3.20	2.45	2.30	4.35	2.80	2.10
Ether extract	4.40	3.95	3.80	2.75	2.15	3.15	2.80	2.90
Ca	0.91	0.91	1.00	0.92	0.95	0.88	0.98	0.93
P	0.76	0.75	0.76	0.67	0.71	0.71	0.72	0.70
Hexanoic acid	0.02	0.10	0.12	0.32	0.47	0.46	0.06	0.03
Octanoic acid	0.01	0.09	0.13	0.33	0.54	0.06	0.44	0.03
Decanoic acid	0.01	0.09	0.15	0.39	0.66	0.03	0.03	0.53
Total MCFA[5]	0.03	0.28	0.40	1.04	1.68	0.54	0.52	0.59
11 to 23 kg								
DM	88.38	88.45	88.19	88.38	87.97	88.67	88.35	88.40
CP	20.90	21.00	21.15	21.10	20.65	20.90	21.50	20.95
ADF	2.80	2.70	2.50	2.35	3.10	3.15	3.05	2.95
Ether extract	3.90	2.90	2.70	2.35	1.90	3.05	3.35	2.90
Ca	0.94	0.87	0.85	0.83	0.84	0.85	0.81	0.85
P	0.69	0.62	0.63	0.65	0.62	0.64	0.64	0.64
Hexanoic acid	0.02	0.07	0.15	0.24	0.50	0.41	0.02	0.01
Octanoic acid	0.01	0.06	0.15	0.29	0.55	0.04	0.37	0.01
Decanoic acid	0.02	0.07	0.18	0.32	0.62	0.02	0.01	0.49
Total MCFA[5]	0.05	0.21	0.48	0.85	1.67	0.47	0.40	0.51

[1] Treatment diets were fed to 360 pigs [DNA 400 × 200 (Columbus, NE); initial BW = 6.7 ± 0.07 kg] for 35-d in a 2-phase feeding program with 5 pigs per pen and 9 pens per treatment.

[2] Sigma Aldrich (St. Louis, MO). MCFA = medium chain fatty acid.

[3] Consisted of a 1:1:1 blend of C6:0, C8:0, and C10:0.

[4] Complete diet samples were collected following feed manufacture using a feed probe to create a composite sample, subsampled, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate and the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for MCFA analysis performed in duplicate. Reported values are average of duplicate analysis.

[5] Sum of analyzed C6:0, C8:0, and C10:0 medium chain fatty acids.

Table 6.3. Effect of dietary addition of medium chain fatty acids (MCFA) on nursery pig growth performance[1]

	Added MCFA, %[2]									Probability, *P*				
		C6:0:C8:0:C10:0[3]				C6:0	C8:0	C10:0						
Item	0	0.25	0.5	1.0	1.5	0.5	0.5	0.5	SEM	Linear[4]	Quadratic[4]	C6:0 vs. control	C8:0 vs. control	C10:0 vs. control
BW, kg														
d 0	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	0.07	0.605	0.641	1.000	1.000	1.000
d 14	10.3	10.6	10.8	11.0	11.2	10.8	11.1	10.5	0.22	< 0.001	0.359	0.178	0.009	1.000
d 35	21.5	22.0	22.7	22.8	23.2	22.5	22.8	22.2	0.34	< 0.001	0.177	0.194	0.033	0.798
d 0 to 14														
ADG, g	255	275	294	305	323	297	314	269	12.9	< 0.001	0.376	0.105	0.007	1.000
ADFI, g	331	333	343	365	376	353	373	325	13.9	0.003	0.994	1.000	0.138	1.000
G:F	0.77	0.83	0.86	0.83	0.86	0.84	0.84	0.82	0.014	< 0.001	0.021	0.004	0.003	0.044
d 14 to 35														
ADG, g	536	544	566	565	572	553	553	555	10.1	0.007	0.245	1.000	1.000	1.000
ADFI, g	829	814	842	868	855	837	834	834	16.8	0.052	0.513	1.000	1.000	1.000
G:F	0.65	0.67	0.67	0.65	0.67	0.66	0.66	0.67	0.008	0.367	0.503	1.000	0.811	0.497
d 0 to 35														
ADG, g	423	436	457	461	472	451	457	441	9.1	< 0.001	0.187	0.159	0.038	0.927
ADFI, g	630	621	642	667	664	644	649	630	14.1	0.010	0.625	1.000	1.000	1.000
G:F	0.67	0.70	0.71	0.69	0.71	0.70	0.70	0.70	0.007	0.004	0.096	0.019	0.005	0.024
Feed PEDV Ct[5,6]														
0 dpi	27.1	29.5	30.9	30.6	32.4	29.7	30.0	28.7	0.27	< 0.001	0.001	< 0.001	< 0.001	0.003
3 dpi	28.3	28.3	29.9	30.9	32.1	30.5	29.5	29.9		< 0.001	0.745	< 0.001	0.061	0.004

[1] A total of 360 pigs [DNA 400 × 200 (Columbus, NE); initial BW = 6.7 ± 0.07 kg] were fed for 35-d in a 2-phase feeding program with 5 pigs per pen and 9 pens per treatment. Bonferroni multiple comparison adjustments were applied to contrasts comparing 0.5% inclusion of individual MCFA compared to control and 0.5% inclusion of individual MCFA compared to 0.5% MCFA blend for all response variables.

[2] Sigma Aldrich (St. Louis, MO).

[3] Consisted of a 1:1:1 blend of C6:0, C8:0, and C10:0.

[4] Linear and quadratic contrast statements include control and treatments fed 1:1:1 blend of MCFA.

[5] Each number is the mean of 3 samples of the 11 to 23 kg diet that were inoculated with porcine epidemic diarrhea virus (PEDV) 40 d following feed manufacturing with a calculated final titer of 10^4 $TCID_{50}$/g feed. On two sampling days post-inoculation [dpi; day of inoculation (0 dpi) and 3 dpi], separate samples were analyzed for the presence of PEDV genetic material using qRT-PCR. Three bottles containing swine feed were not inoculated with PEDV as a negative control and analyzed on d 0, and qRT-PCR analysis did not detect genetic material using a threshold cutoff value of 45 cycles. A higher Ct value indicates a greater amount of PEDV genetic material present in the original sample. SEM representative for entire qRT-PCR analysis.

[6] Treatment × day, $P < 0.001$; Quadratic inclusion of MCFA blend × day, $P = 0.023$; 0 dpi C6:0 vs. 0.5% 1:1:1 and 0.5% C10:0 vs. 0.5% 1:1:1, $P \leq 0.047$. SEM represents both 0 and 3 dpi.

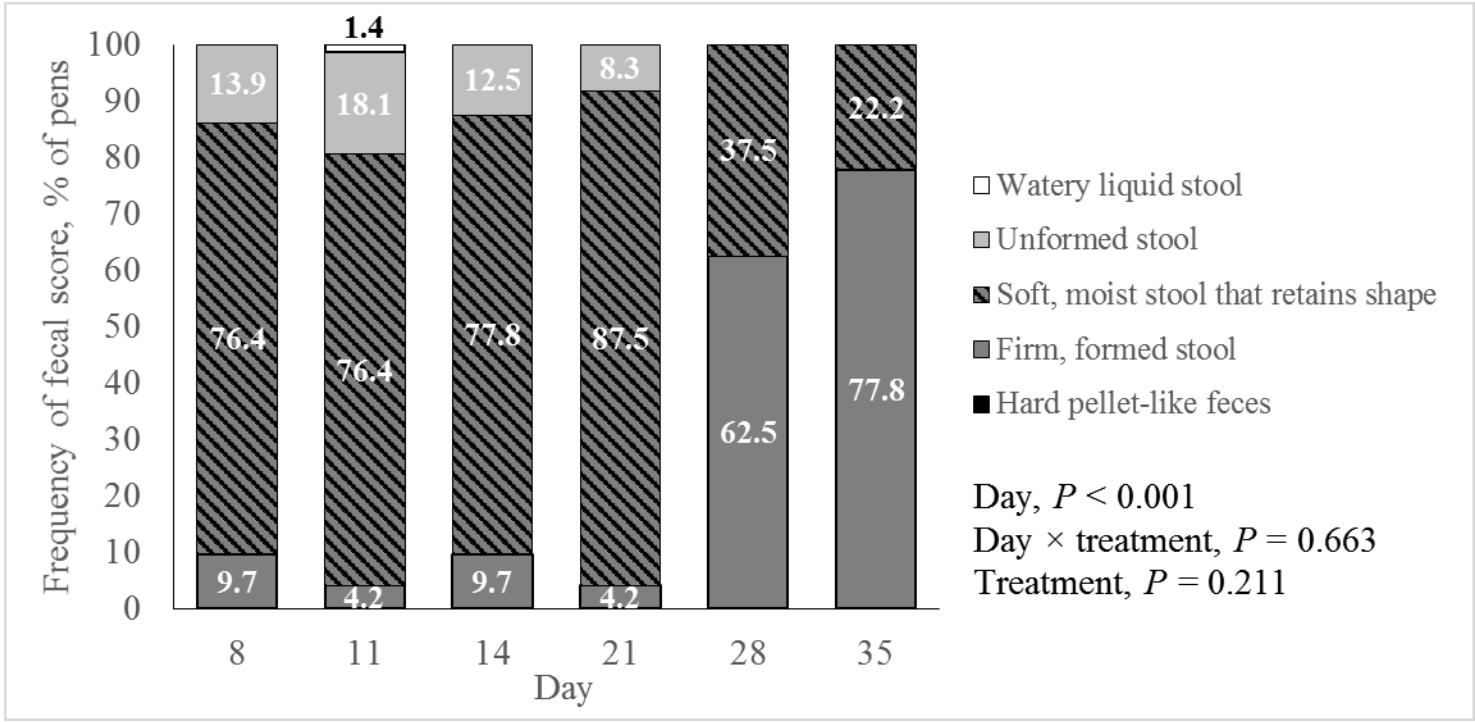

Figure 6-1. Effects of study day on fecal consistency of nursery pigs assessed by fecal score. Graph bars show the frequency distribution of fecal scores (n = 72 pens) within each fecal score category according to day regardless of dietary treatment. Score was evaluated by 3 trained individuals on each day and the concordant score was considered the definite score for each pen on each day of evaluation.

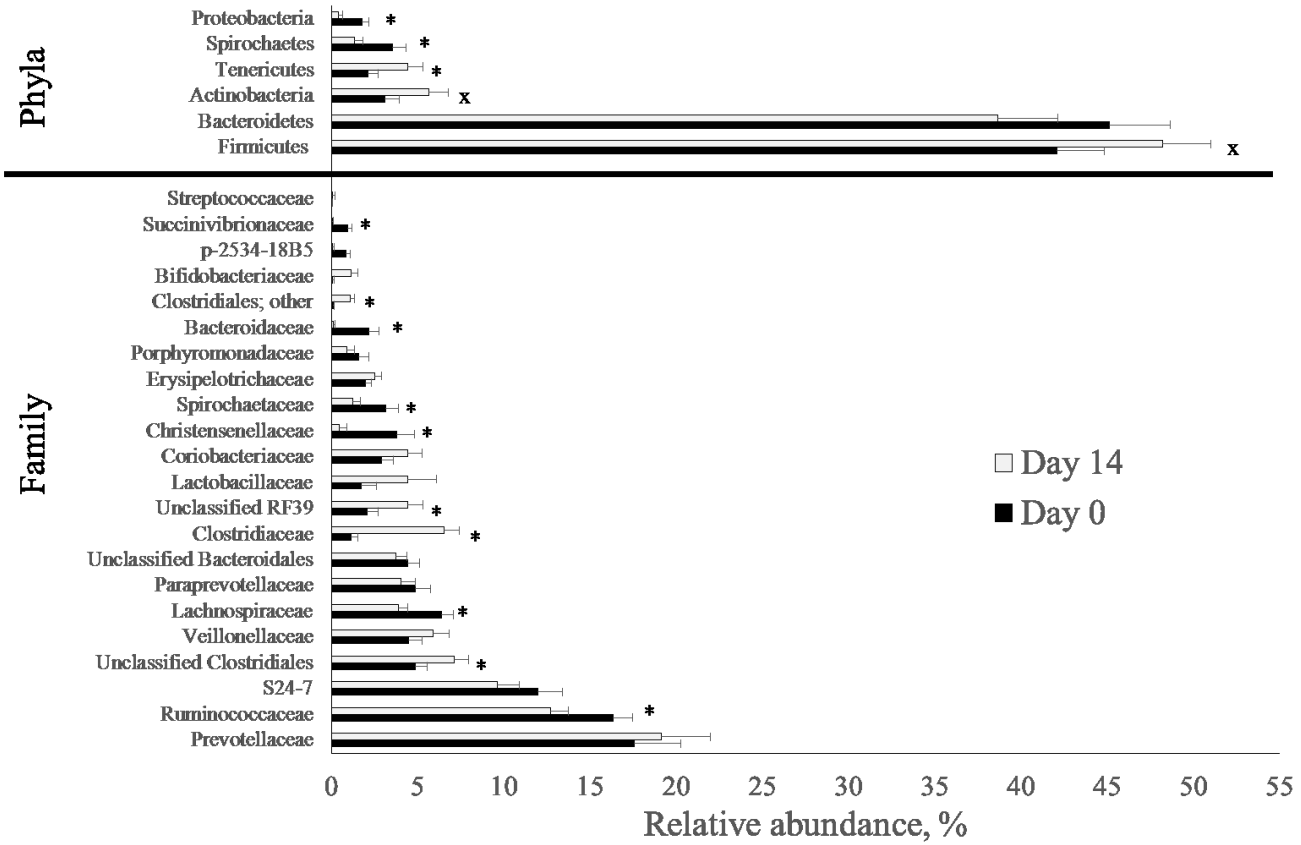

Figure 6-2. Relative abundance of microbial phyla and families by day presented as proportion of all reads for a specific sample classified into the designated microbial phyla or family ± SEM. A total of 18 pigs (1 pig per pen from each of 9 control diet-fed pens and 1 pig per pen from each of 9 pens fed 1.5% medium chain fatty acid (MCFA) blend) were selected randomly for sampling of fecal material for characterization of fecal microbial populations. Blend of MCFA consisted of 1.5% blend of 1:1:1 ratio of C6, C8, and C10; Sigma Aldrich (St. Louis, MO). Sampling of the same set of pigs occurred on d 0 and d 14. Samples were analyzed using 16s rDNA sequencing. Microbial phyla and families lacking at least one treatment × day combination having a relative abundance of ≤ 1% are not shown. A dietary treatment × day interaction was observed for Proteobacteria ($P = 0.057$), where relative abundance was consistent over time in pigs fed the control diet ($P = 0.668$), whereas a decrease over time was observed in pigs fed the 1.5% MCFA blend diet ($P = 0.023$). All other treatment × day, $P \geq 0.138$. *Main effect of day, $P < 0.05$; ^{x}Main effect of day, $0.05 < P \leq 0.10$.