USPCE and NPB Swine Research Experience & Educations (SREE) FINAL RESEARCH GRANT REPORT FORMAT

As a requirement of each Swine Research Experience & Education (SREE) funded, a final report detailing the project results must be provided to the U.S. Pork Center of Excellence and the National Pork Board. The content of the report can be written for a scientific audience.
ALL final reports must be submitted in the following format or the report will be returned for correction. PLEASE DO NOT INCLUDE ANY TYPE OF COVER PAGE.

Project Title: Effect of corn particle size on gestating sow fecal microbiome and volatile fatty acid production

Principal Investigator: Rachel Kort
Principal Instructor and Title: Dr. Chad Paulk, Associate Professor
Institution: Kansas State University
Date Report Submitted: March 31, 2021

Keywords: gestating sows, particle size, volatile fatty acids, fecal microbiome

Scientific Abstract:
Previous research has demonstrated that for every 100 µm reduction in corn particle size finishing pig feed efficiency is improved 1.0 to 1.3%. This improvement results from an increase in concentration of metabolizable energy in corn. However, limited data is available on the effects of corn particle size when fed to gestating sows and the subsequent effects on fecal microbiome and volatile fatty acid (VFA) production. Therefore, the objective of this study was to determine the effect of corn particle size on gestating sow fecal microbiome and VFA production. Three particle sizes (400, 800, and 1200 µm) of corn were mixed into a complete gestation diet and fed to gestating sows (9 sows per treatment). Feces were then collected and analyzed to determine microbiome concentrations and VFA production. Sows fed diets with decreasing corn dgw had increased (quadratic, $P = 0.021$) fecal acetic acid proportions, and decreased propionic (quadratic, $P = 0.019$) and valeric acid (linear, $P = 0.005$). There was no evidence of difference in total VFA production or molar proportions of isobutyric acid, butyric acid, isovaleric acid, or caproic acid. After quality filtering, the dataset consisted of 1,181,605 reads with 111 taxa. Analysis was rarefied to an even depth to ensure no bias and used for subsequent alpha diversity analysis. Alpha diversity was investigated using both observed amplicon sequence variants (ASV) and Shannon even richness measures. Results showed no significant differences between treatments for observed ASVs. However, there was a significant difference between sows fed 400 µm and 1,200 µm using Shannon indexes ($P = 0.017$). For additional analysis, data was analyzed based off proportions. Globally, beta diversity was investigated using a weighted UniFrac distance matrix to investigate differences among treatments. Additionally, a PERMANOVA analysis was performed using main effects of treatment ($P = 0.007$), parity, acetate, propionate, isobutyrate, butyrate, valerate, and caproic acids. However, no other significant effects were detected for parity, acetate, propionate, isobutyrate, butyrate, valerate, and caproic ($P = 0.396$, $P = 0.051$, $P = 0.188$, $P = 0.618$, $P = 0.870$, $P = 0.425$, $P = 0.598$, respectively). Eight differential ASVs were identified between treatment diets using DESeq2. Interestingly, ASV_25_*Papillibacter* was in high abundance for sows fed 400 and 800 µm corn but in low abundance for sows fed 1,200 µm corn. Likewise, ASV_95 *Clostridium sensu stricto* 1 showed low abundance in sows fed 400 µm corn but was in higher abundance in treatment 800 and 1,200 µm corn. In conclusion, it was hypothesized that feeding gestating sows corn ground to a coarser particle size would result in a shift in the

hindgut bacterial population and VFA production that could improve health for gestating sows. However, there was no evidence of difference in total production or molar proportion of butyrate in fecal samples collected from gestating sows.

Introduction:

An overview of the researchable question and its importance to producers.
The goal of feeding gestating sows is to support growth and development of fetus and sows, support rapid fetal growth beginning in mid- to late gestation and mammary development. Gestating sows are limit fed to control weight gain and therefore dietary energy is a primary focus of diet formulation for gestating sow. Previous research has demonstrated that reducing corn particle size leads to a 1.0 to 1.3% improvement in finishing pig feed efficiency for every 100 µm reduction in particle size when ground from approximately 1,000 to 400 µm (Cabrera, 1995; Wondra, 1995; Paulk, 2011; De Jong 2012). This improvement results from an increase in concentration of ME in corn as particle size is reduced (Rojas and Stein; 2015). Wondra et al. (1995) observed a 1.3% improvement in litter weight gain for 100 µm reduction in corn particle size fed to lactating sows. Although there are improvements in performance of lactating sows, fine grinding of grains has been shown to increase the incidence of ulceration of the pars esophageal region of the stomach (Wondra et al. 1995), potentially increasing mortality. Therefore, particle size recommendations for gestating sows are coarser than those for lactation, nursery, and finishing pigs. However, there is limited data to support this recommendation. When determining the optimal particle size for gestating sows, it is important to consider the effects on energy, health, and longevity of the sow.

Gestating sow health has previously been indicated by the effects of grain particle size on stomach ulcers (Wondra et al., 1995). However, other indicators of sow health such as the potential benefits of volatile fatty acid (VFA) substrates in the large intestine have not been measured. Particle size of grain can influence the material that is undigested and passed through the small intestines into the large intestines of pigs. An increase in substrates in the large intestines can potentially lead to changes in the microbiome and nutrients produced. Previous research has determined that an increased production of butyrate in the large intestine can serve as a primary nutrient that provides energy to colonocytes. Butyrate can function as acellular mediator regulating multiple functions of gut cells, including gene expression, cell differentiation, gut tissue development, immune modulation, oxidative stress reduction, and diarrhea control (Bedford and Gong, 2017). Therefore, it is hypothesized that feeding gestating sows corn ground to a coarser particle size will result in a shift in the hindgut bacterial population and volatile fatty acid production that would be indicative of improved health for gestating sows.

Objectives:

To evaluate the effects of corn particle size on gestating sow fecal microbiome and volatile fatty acid production.

Materials & Methods:

Kansas State University Institutional Animal Care and Use Committee approved all protocols used in this trial. This experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS from mid to late February 2020.

Animal Housing, Diet, and Feeding

A total of 27 mixed parity gestating sows (Line 241; DNA, Columbus, NE) were used during the second phase of gestation (d 61-82). Sows were individually housed in an environmentally controlled room with mechanical ventilation. Sows had *ad libitum* access to water via nipple waterer. Sows were fed 2, 2.5, or 3 kg once daily at 0700 based on a body condition of 2, 3, or 4 respectively. A corn-soybean meal-based diet

(Table 1) was formulated based on the NRC (2012) recommendations. There were no ingredient inclusion changes across treatments. Diets consisted of corn ground to either 400, 800, or 1200-µm using a 3 high roller mill (Model 924, RMS Roller Grinder, Harrisburg, SD). Sows were split into 3 groups based upon breeding date and allotted to dietary treatment within group and balanced by parity and back fat. Titanium dioxide (0.25%) was added to the diets as an indigestible marker. Sows were fed for 21 d to allow for adaptation to the treatment diets followed by a two-day collection period of fecal and urine samples.

Sample Collection

Fecal grab samples were collected twice daily for 2 d, bagged, and stored separately at -20 °C. At the end of the trial samples were pooled within sow and subsamples for analysis. All samples were stored at -80°C until samples were sent to the University of Georgia for analysis of VFA concentrations and to the University of Nebraska- Lincoln for fecal microbiome analysis.

Sample Analysis

To determine particle size of both the corn used in the diets, and the diets the RoTap 13-sieve method utilizing 0.5g sieving agent with a 10-minute run time was used (ANSI/ASAE method S319.2,1996). Data were analyzed using the PROC Glimmix procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) utilizing linear and quadratic polynomial contrasts with sow as the experimental unit and treatment as the fixed effect. Results were considered significant if $P \leq 0.05$ and marginally significant if $P \leq 0.10$.

Analysis of VFAs were performed according to the procedure described in Lourenco et al. (2020[1]). One gram of feces was diluted with 3 mL of distilled water and placed into 15-mL conical tubes. The tubes were vortexed for 30 seconds to produce a homogeneous sample and 1.5 mL of the mixture was transferred to microcentrifuge tubes. The tubes were centrifuged at 10,000 x g for 10 minutes. One mL of the supernatant was transferred into a new microcentrifuge tube and mixed with 0.2 mL of metaphosphoric acid solution (25% w/v). The samples were vortexed for 30 seconds and stored at -20°C overnight. The next morning, samples were thawed and centrifuged at 10,000 x g for 10 minutes. The supernatant was removed and transferred into polypropylene tubes combined with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant. Tubes were vortexed for 10 seconds to thoroughly mix them and allowed to settle for 5 minutes for optimum separation. Then 600 µL of the top layer was transferred into screw-thread vials. Analysis for VFAs were performed using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector and a capillary column (Zebron ZB-FFAP; 30 m x 0.32 mm x 0.25 µm; Phenomenex Inx., Torrance, CA, USA). Sample injection volume was set to 1.0 µL, and helium was used as a carrier gas. Column temperature started at 110°C and increased to 200°C over the course of 6 minutes. The injector temperature was set to 250°C , and the detector temperature was set to 350°C.

Fecal Microbiome

Collected fecal samples were extracted using the Omega Soil Mag-Bind 96 Well Kit (Omega Bio-Tek Inc, Norcross, GA, USA) as described by manufacturer. A single modification was added prior to isolating the DNA by implementing a precipitation step as previously described by Yu and Morrison (2003) and Paz et al., (2018). Extracted DNA quality was assessed using a gel electrophoresis and the resulting non-degraded DNA was stored at -20°C until used for bacterial community analysis. The V4 region of the 16S rRNA gene was amplified using barcoded universal primers specific to the V4 region in 25 µl PCR reactions as described previously (Kozich et al., 2013). The PCR reactions contained 0.625 Units of Terra PCR Direct Polymerase Mix (Clontech Laboratories Inc., Mountain View, CA, USA), 1X Terra PCR Direct Buffer (Clontech Laboratories Inc., Mountain View, CA, USA), 0.4 uM indexed primers, and 20-50ng of total DNA.

Amplifications were performed on a Veriti 96-Well Thermocycler (Life Technologies, Carlsbad, CA, USA) with conditions of 98°C for 2 min followed by 25 cycles of 98°C for 30 s, 58°C for 30 s, 68°C for 45 s, with a final extension of 68°C for 4 min. Resulting amplicons were normalized using NGS Normalization 96-Well Kit (Norgen BioTek Corp., Thorold, ON, Canada) according to the manufactures instructions with the addition of a 5 min spin at 3,200× g before the elution buffer was added. Amplicons were pooled and concentrations of pooled samples were measured using a Denovix Fluorescence High Sensitivity Assay (Denovix Inc., Wilmington, DE, USA). Resulting libraries ready to sequence were further analyzed using the Agilent BioAnalyzer 2000 (Agilent Technologies, Santa Clara, CA, USA). All libraries were sequenced using the 250bp paired end sequencing strategy using the Illumina MiSeq platform with a V2 500 cycle sequencing kit as described by the manufacturer (Illumina, San Diego, CA, USA). The sequencing run included six negative controls to remove any reagent associated contaminants. Additionally, one positive control of a known mock community was included to ensure proper sequencing depth and read threshold. One sample was removed from the study due to poor DNA quality and sequence read depth.

Data analysis of the reads generated was performed using the DADA2 pipeline (Callahan et al., 2016). Briefly, the fastq files generated above were quality filtered and was used to identify amplicon sequence variants (ASVs). Analytical steps were performed using R v1.1.463 (R Core Team, 2019) using the phyloseq package v1.26.1 (McMurdie and Holmes, 2013). All low-quality reads were filtered (Q score $\geq$ 30) and trimmed. "Unique sequences" were identified by combining identical sequences and error rates that were estimated to evaluate read quality. Forward and reverse reads were assembled to generate contigs for the V4 region. Additional quality filtering was performed to remove sequences with ambiguous bases, incorrect contig lengths, and chimeras. The SILVA reference alignment database v138 was used in conjunction with MOTHUR (v.1.42.1; Schloss et al., 2009) to generate a phylogenetic tree. The resulting phylip.tree, sequence tables, mapping files, and taxonomy tables were merged to create a "phyloseq object" and was used for subsequent analysis. Negative controls were used to remove any contaminants which may have arisen through reagents. Likewise, a positive control was used to identify read abundances based on known positive control inputs.

Statistical analysis was performed using the adonis function in the 'R' package 'vegan' (Dixon, 2003)[8]. The PERMANOVA analysis was performed using a weighted UniFrac distance matrix to identify factors affecting microbial community structure. Shannon and observed ASVs were used to investigate alpha diversity. Additionally, a Wilcoxon rank sum test (RC, 2020)[9] was used to evaluate any statistical differences. The DESqeq2 function was used to identify differential ASVs present between diets using the Benjamini error correction method (Huber, 2010). The BGLR function was used in 'R' to predict regression differences between VFAs. All significance was set to $P < 0.05$

Results:

Volatile Fatty Acid Production

Sows fed diets with decreasing corn geometric mean diameter (d_{gw}) had increased (quadratic, $P = 0.021$) fecal acetic acid proportions, and decreased propionic (quadratic, $P = 0.019$) and valeric acid ($P = 0.005$; Table 2) proportions. However, there was no evidence of differences in total VFA production or molar proportions of isobutyric acid, butyric acid, isovaleric acid, or caproic acid.

Fecal Microbiome

After quality filtering, the dataset consisted of 1,181,605 reads with 111 taxa. Analysis was rarefied to an even depth to ensure no bias and used for subsequent alpha diversity analysis. Alpha diversity was investigated using both observed ASVs and Shannon even richness measures. Results showed no significant

differences between treatments for observed ASVs. However, there was a significant difference between sows fed 400 µm and 1,200 µm using Shannon indexes (P = 0.017; Figure 1). For additional analysis, data was analyzed based off proportions. Globally, beta diversity was investigated using a weighted UniFrac distance matrix to investigate differences among treatments (Figure 2). Additionally, a PERMANOVA analysis was performed using main effects of treatment, parity, acetate, propionate, isobutyrate, butyrate, valerate, and caproic. Significant differences were detected for treatment (P = 0.007). However, no other significant effects were detected for parity, acetate, propionate, isobutyrate, butyrate, valerate, and caproic (P = 0.396, P = 0.051, P = 0.188, P = 0.618, P = 0.870, P = 0.425, P = 0.598, respectively).

The DESeq2 function was used to identify differential ASVs between treatment diets. Eight differential ASVs were identified (Figure 3). Interestingly, ASV_25_Papillibacter was in high abundance for sows fed 400 and 800 µm corn but in low abundance for sows fed 1,200 µm corn (Figure 3). Likewise, ASV_95_Clostridium_sensu_stricto_1 showed low abundance in sows fed 400 µm corn but was in higher abundance in treatment 800 and 1,200 µm corn.

Discussion: Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

This project was done in conjunction to Nichols, 2020, which determined that the reduction of corn particle size from 1200 to 400 µm resulted in a 5% improvement in crude protein digestibility and a 6% improvement in dietary gross energy digestibility. Therefore, reducing the corn particle size resulted in a reduction of the flow of nutrients to the large intestines to be utilized by microbes. However, there was no evidence of difference in the overall production of total VFAs. Decreasing corn particle size led to an increase in the proportion of acetic acid and a decrease in propionic and valeric acid in fecal samples of gestating sows. Previous research has determined that an increased production of butyrate in the large intestine can serve as a primary nutrient that provides energy to colonocytes and is a cellular mediator regulating multiple functions of gut cells, including gene expression, cell differentiation, gut tissue development, immune modulation, oxidative stress reduction and diarrhea control (Bedford and Gong, 2017). Additionally, microbiome analysis showed there was a significant difference between sows fed diets containing corn ground to 400 µm compared to 1,200 µm. Eight differential ASVs were identified. Of which, 25% belonged to the family *Clostridiaceae*, 25% belonged to Lachnospiraceae, and another 25% belong to *Oscillospiraceae*. Interestingly, ASV_25_ *Papillibacter* were in greater abundance for sows fed corn ground to 400 and 800 µm compared to 1,200 µm. *Papillibacter* belongs to the family *Oscillospira* and is rarely isolated, although it is commonly found in the gastrointestinal tract (Chen et al., 2020). According to Gophna et al., (2017), inferred that *Oscillopsira* species are butyrate producers and are often observed to be reduced in the presence of diseases that cause inflammation. As such, butyrate is a major source of energy for colonocytes (Chen et al., 2015 & Jacobi and Olde 2012) and modulates biological responses for gastrointestinal health (Liu et al., 2018). Likewise, colonocytes are thought to contribute to maintain homeostasis by shaping the microbiota to be beneficial. Interestingly enough, *Oscillospira*, a butyrate producer, has been reported to cause constipation at high concentrations (Chen et al., 2020). Yet it is also reported that while butyrate helps control inflammation, at high concentrations it can disrupt the intestinal barrier (Liu et al., 2018). Thus, this might be indicative of the changes in abundances seen in this experiment with ASV_25_*Papillibacter* between the three diets. Therefore, it was hypothesized that feeding gestating sows corn ground to a coarser particle size would result in a shift in the hindgut bacterial population and VFA production that would be indicative of improved health for gestating sows. However, there was no evidence of difference in total production or molar proportion of butyrate in fecal samples collected from gestating sows.

Citations

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP: DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016, 13(7):581-583.

Chen J, Li Y, Tian Y, Huang C, Li D, Zhong Q, Ma X. Interaction between Microbes and Host Intestinal Health: Modulation by Dietary Nutrients and Gut-Brain-Endocrine-Immune Axis. Curr Protein Pept Sci. 2015;16(7):592- 03. doi: 10.2174/1389203716666150630135720. PMID: 26122779.Chen, Yr., Zheng, Hm., Zhang, Gx. et al. High Oscillospira abundance indicates constipation and low BMI in the Guangdong Gut Microbiome Project. Sci Rep 10, 9364 (2020). https://doi.org/10.1038/s41598-020-66369-z

Dixon P: VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 2003, 14.

Gophna U, Konikoff T, Nielsen HB. Oscillospira and related bacteria - From metagenomic species to metabolic features. Environ Microbiol. 2017 Mar;19(3):835-841. doi: 10.1111/1462-2920.13658. Epub 2017 Feb 3. PMID: 28028921.

Huber SAW: Differential expression analysis for sequence count data. Genome Biology 2010, 11.

Jacobi SK, Odle J. Nutritional factors influencing intestinal health of the neonate. Adv Nutr. 2012 Sep 1;3(5):687-96. doi: 10.3945/an.112.002683. PMID: 22983847; PMCID: PMC3648750.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD: Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 2013, 79(17):5112-5120.

Liu H, Wang J, He T, Becker S, Zhang G, Li D, Ma X. Butyrate: A Double-Edged Sword for Health? Adv Nutr. 2018 Jan 1;9(1):21-29. doi: 10.1093/advances/nmx009. PMID: 29438462; PMCID: PMC6333934.

Lourenco J. M., Kieran T. J., Seidel D. S., Glenn T. C., da Silveira M. F., Callaway T. R., Stewart R. L. Comparison of the ruminal and fecal microbiotas in beef calves supplemented or not with concentrate. PLOS ONE (2020) 15: e0231533. doi: 10.1371/journal.pone.0231533.

McMurdie PJ, Holmes S: phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013, 8(4):e61217.

Paz HA, Hales KE, Wells JE, Kuehn LA, Freetly HC, Berry ED, Flythe MD, Spangler ML, Fernando SC. Rumen bacterial community structure impacts feed efficiency in beef cattle. J Anim Sci. 2018 Apr 3;96(3):1045-1058. doi: 10.1093/jas/skx081. PMID: 29617864; PMCID: PMC6093515.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ et al: Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009, 75(23):7537-7541.

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

Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques. 2004 May;36(5):808-12. doi: 10.2144/04365ST04. PMID: 15152600.

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

Ingredient, %	Gestation diet
Corn	78.14
Soybean meal, 46.5% crude protein	17.29
Soy oil	0.50
Monocalcium phosphate, 21% P	1.30
Limestone	1.30
Salt	0.50
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.25
Sow add pack[4]	0.25
Phytase[5]	0.08
Titanium dioxide	0.25
Total	100.0

continued

Table 1. Diet composition (as-fed basis)

Calculated analysis5, as-fed basis:	
Metabolizable energy, kcal/kg	3,265
Net energy, kcal/kg	2,486
Crude protein, %	14.7
Ca, %	0.91
P, %	0.61
Ca:P	1.50
Analyzed nutrients, as-fed basis	
Dry matter, %	89.40
Gross energy, kcal/kg	3887
Crude protein, %	14.74

1 Gestation diets were fed during the second phase of gestation (d 61-82) for 21 d to allow for adaptation to the treatment diets followed by a 2 d collection period. Dietary treatments consisted of corn ground to either 400, 800, or 1200-µm with titanium dioxide (0.25%) as an indigestible marker.

[2] Provided per kg of premix: 3 g Cu from copper sulfate; 160 mg Ca from calcium iodate; 31 mg Fe from ferrous sulfate; 3 g Mn from manganese sulfate; 120 mg Se from sodium selenite; and 31 g Zn from zinc sulfate.

[3] Provided per kg of premix: 1,543,220 IU vitamin A from vitamin A acetate; 440,920 IU vitamin D from vitamin D3; 8,047 IU vitamin E from dl-α-tocopherol acetate; 882 mg menadione from menadione nicotinamide bisulfite; 8 mg B12 from cyanocobalamin; 14,991 mg niacin from niacinamide; 6,614 pantothenic acid from d-calcium pantothenate; 1,984 mg riboflavin from crystalline riboflavin.

[4]Provided per kg of premix: 0.077 g chromium, 1,653,750 IU vitamin A from vitamin A acetate; 8,820 vitamin E from dl-α-tocopherol acetate; 88.2 mg biotin, 882 mg Folic acid, 367 mg Pyridoxine, 220,500 mg Choline, 19,845 mg Carnitine.

[5] Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 1,102,300 phytase units (FTU)/kg of product with a release of 0.10% available P.

[6] Calculated nutrient values are based on NRC (2012).

Table 2. The effect of corn particle size on diet and corn energy value and the total concentration and the molar proportions of volatile fatty acids (VFA) in the feces of gestating sows.[1]

	Particle size, µm				Probability, *P* <	
Item	400	800	1200	SEM	Linear	Quadratic
Corn, geometric mean diameter (d_{gw})[2]	404	823	1372	-	-	-
Diet, d_{gw}[2]	448	733	1155	-	-	-
Total VFA (mmol/L)[3]	117	110	151	16.5	0.155	0.228
Molar proportions[3]						
Acetic acid	0.646	0.670	0.560	0.0230	0.014	0.021
Propionic acid	0.175	0.170	0.248	0.0140	0.001	0.019

Isobutyric acid	0.018	0.020	0.021	0.0013	0.090	0.854
Butyric acid	0.084	0.065	0.082	0.0102	0.871	0.132
Isovaleric acid	0.046	0.054	0.058	0.0056	0.063	0.704
Valeric acid	0.016	0.016	0.026	0.0023	0.005	0.094
Caproic acid	0.013	0.539	0.537	0.0046	0.120	0.345

[1]A total of 27 mixed parity gestating sows (Line 241; DNA, Columbus, NE) were used during the second phase of gestation (d 61-82). Sows were individually housed and split into 3 groups based on breeding date and allotted to dietary treatment within group, balanced by parity and back fat. Sows were fed for 21 d to allow for adaptation to the treatment diets followed by a 2 d collection period. Sows were fed 2, 2.5, or 3 kg once daily at 0700 based on a body condition score of 2, 3, or 4, respectively. Dietary treatments consisted of corn ground to either 400, 800, or 1200-µm with titanium dioxide (0.25%) as an indigestible marker.

[2]ANSI/ASAE method S319.2,1996.

[3]Analysis of VFAs were performed according to the procedure described in Lourenco et al. (2020).

Figure 1. The effects of corn particle size (A = 400 µm, B = 800 µm, and C = 1,200 µm) on changes in alpha diversity in fecal samples from gestating sows. Presented are the observed amplicon sequence variants (ASVs) and Shannon estimates. Significant differences were identified in alpha diversity between 400 µm and 1,200 µm (*P* = 0.017; Shannon).

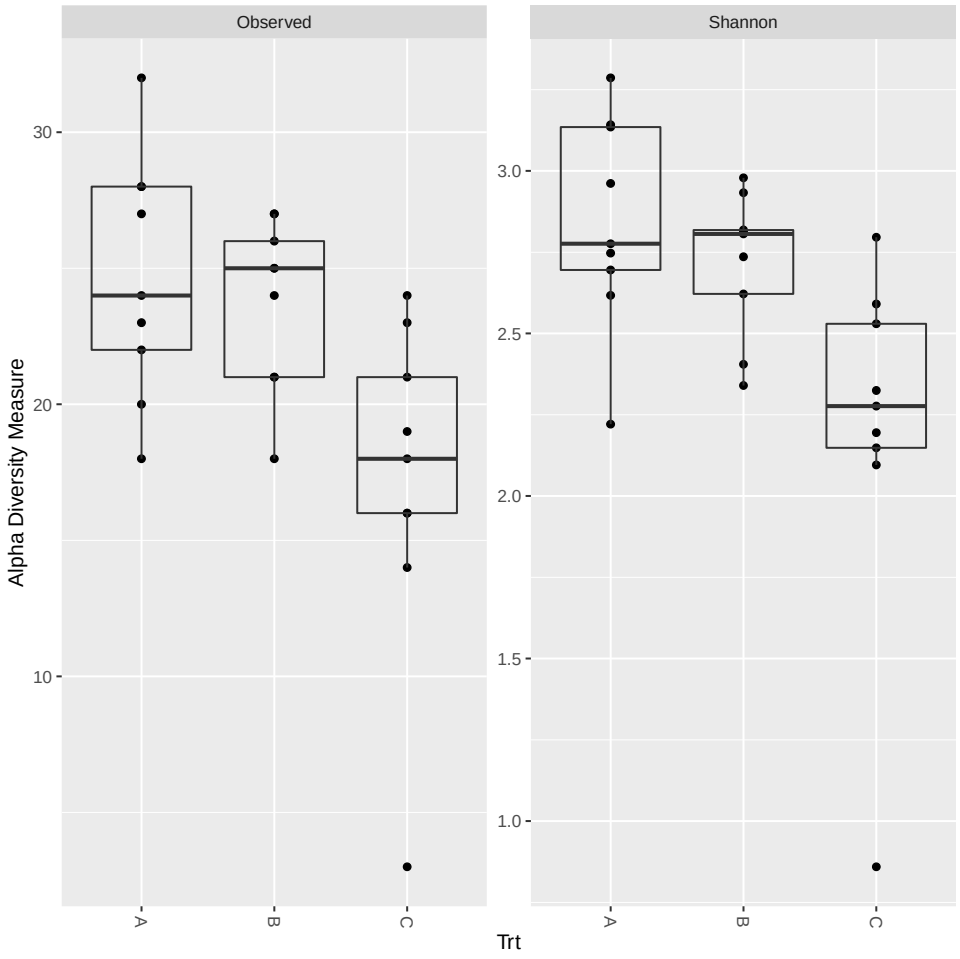


Figure 2. The effects of corn particle size (A = 400 µm , B = 800 µm , and C = 1,200 µm) on changes in Principal Coordinate Analysis (PCoA) demonstrating variations in fecal samples collected from sows. The PCoA was generated using a weighted UniFrac distance matrix. The PERMANOVA analysis demonstrated microbial communities to be significantly different based on diet (*P* = 0.007).

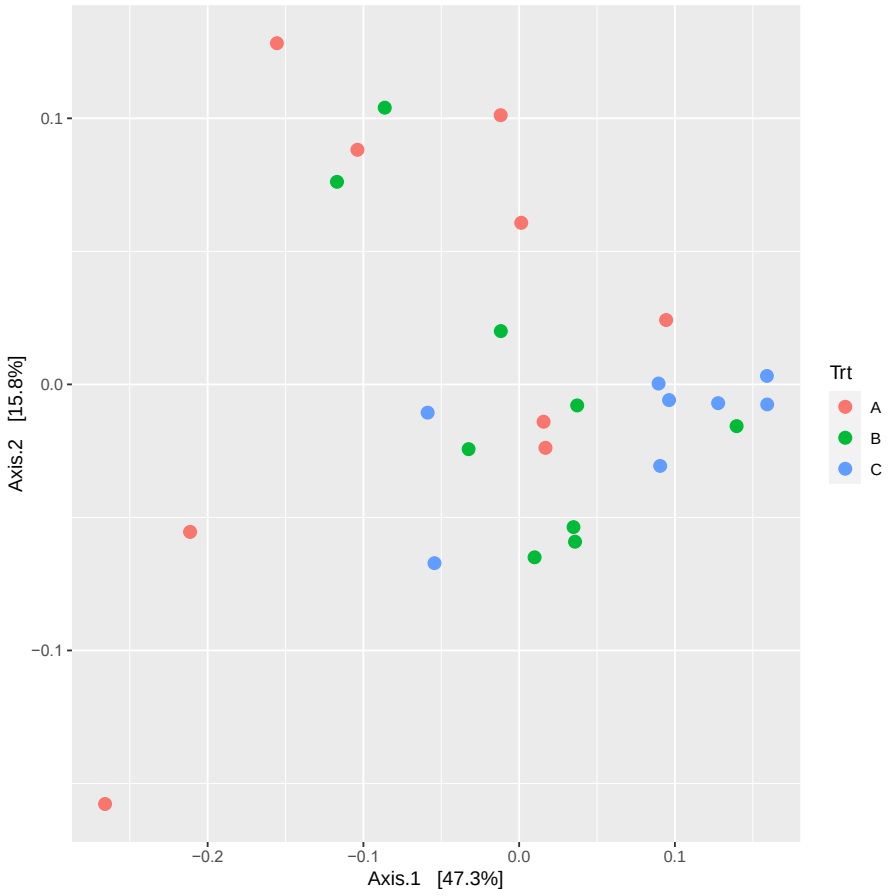
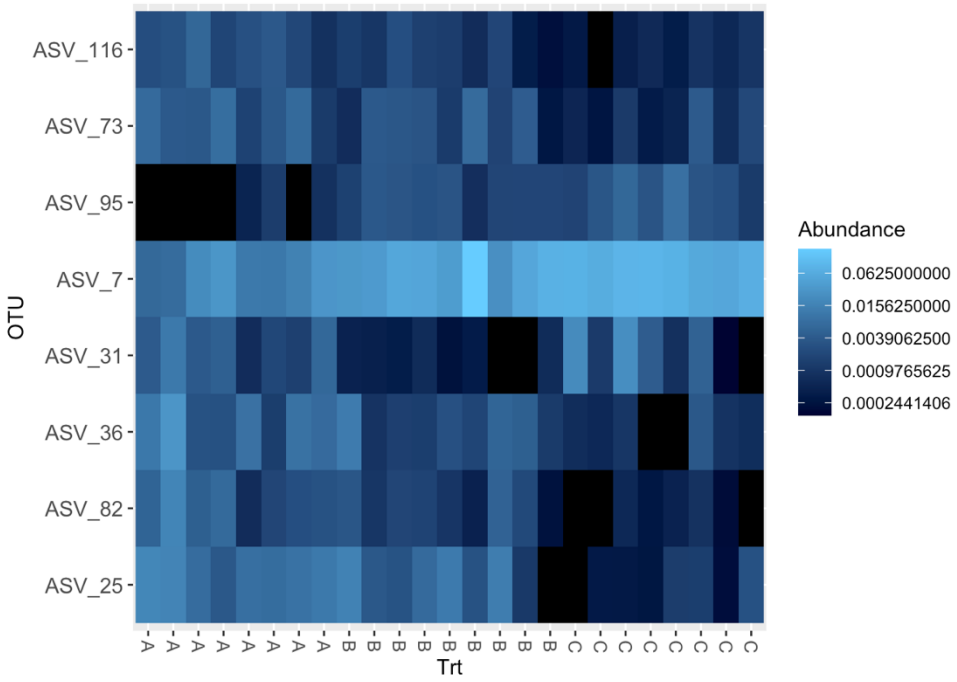


Figure 3. The effects of corn particle size (A = 400 µm , B = 800 µm , and C = 1,200 µm) on changes in heat-map showing distribution of differential amplicon sequence variants (ASVs) alpha diversity in fecal samples from gestating sows. Major differences were driven by ASV_25_*Papillbacter*.



Student Statement: Write a brief summary of how your Swine Research Experience & Education affected you. Please include what you learned, if you presented your results anywhere, and how the research funded affects your future endeavors or studies. Also, include any future plans you have after you obtain your degree.

My Swine Research Experience & Education project was a huge building block in helping establish my interest in the swine industry. This project allowed me to dig deeper into previous studies that had been conducted and different factors that affect sow growth. I learned all the key components of research, including developing a hypothesis, designing an experiment, conducting the experiment, caring for sows, data analysis, data interpretation, and presentation. The focus of this project allowed me to explore and learn more about how feed manufacturing influenced different responses when feeding gestation sows. It was exciting to connect the dots between my major in feed science and management and my interest in attending vet school. I presented this project at the 2021 American Society of Animal Science- Midwest Section scientific meeting in the Undergraduate Oral Competition. This project help solidify my interest in the swine industry and further pursue my career in veterinary medicine focusing on swine. I will be attending the University of Nebraska-Lincoln this coming August to begin in the Iowa State University Veterinary Medicine 2+2 Program. After vet school, I plan on becoming a swine vet for an industry driven company. Research, like this project, will allow me to help make advancements in the swine industry throughout my professional career.