Impact of feed additives in swine diets and the effect of corn particle size on metabolizable energy in gestating sows.

by

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Abstract

One experiment was conducted to evaluate the effect of formic acid and lignosulfonate addition on pellet quality. A second and third experiment were conducted to analyze the effect of 5 feed additives on PEDv and PRRSv in a feed matrix. A fourth study was conducted to analyze the effect of corn particle size on digestible and metabolizable energy in gestating sows. In experiment 1, 5 treatments consisting of a control, or the control with 2 levels of formic acid (0.36 % and 0.6%), or the control with a blended product containing 60% formic acid and 40% lignosulfonate (blended product was included at 0.6% and 1.0% of the diet). Diets were pelleted and analyzed pellet durability and hardness. Increasing formic acid in the diet decreased pH ($P < 0.001$). No evidence for differences ($P > 0.05$) were observed for pellet mill energy consumption, or pellet durability, regardless of testing method or pellet hardness when adding formic acid or lignosulfonate to the diet. In experiment 2, 12 chemical treatments 1) non-treated, individually inoculated virus controls (positive control), 2) 0.33% commercial formaldehyde-based product (Sal Curb; Kemin Industries, Inc.; Des Moines, IA), 3) 0.50% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO), 4) 0.25%, 5) 0.50%, or 6) 1.00% of commercial dry mono and diglyceride-based product (Furst Strike; Furst-McNess Company, Freeport, IL), 7) 0.25%, 8) 0.50%, or 9) 1.00% of commercial dry mono and diglyceride-based product (Furst Protect; Furst-McNess Company, Freeport, IL), 10) 0.25%, 11) 0.50%, or 12) 1.00% dry mono and diglyceride-based experimental product (Furst-McNess Company, Freeport, IL). In total there were 12 treatments with 3 replications per treatment. A complete swine feed was treated with each chemical treatment before inoculation with 10^6 $TCID_{50}$/g of feed with PEDV or PRRSV. PEDV or PRRSv RNA detection levels were then analyzed via quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR). Formaldehyde

and MCFA decreased ($P < 0.05$) the detectable RNA concentration of PEDV and PRRSv compared to all other treatments. Furst Strike, Furst Protect, and the experimental product did not reduce detectable concentrations. In experiment 3, 4 chemical treatments were used 1) non-treated, individually inoculated virus controls (positive control), 2) 0.33% commercial formaldehyde-based product (Sal Curb; Kemin Industries, Inc.; Des Moines, IA), 3) 0.50% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO). In total there were 4 treatments with 3 replications per treatment. A complete swine feed was treated with each chemical treatment before inoculation with 10^6 $TCID_{50}$/g of feed with PEDV and PRRSv or a combination of the two. Sal Curb and MCFA both increased ($P < 0.05$) feed Ct values in the co-infection treatments for both viruses, whereas only Sal Curb increased Ct values when used against PRRSv ($P < 0.05$). The fourth and final experiment evaluated the effect of corn particle size on energy digestibility in gestating sows. 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). Feed, feces, and urine were then analyzed for energy, nitrogen, and titanium dioxide levels to calculate digestible, metabolizable and nitrogen corrected metabolizable energy. Crude Protein, Digestible and Metabolizable energy (ME), nitrogen adjusted ME and Corn ME all increased (linear, $P < 0.05$) as particle size was reduced from 1200 to 400 μm.

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Dedication

This thesis is dedicated to my late grandfather Jack, for always pushing me to find my passion and pursue it relentlessly.

Chapter 1 - Effect of Dietary Formic Acid and Lignosulfonate on Pellet Quality

Abstract

The objective of this study was to determine the effect of formic acid and lignosulfonate (LignoTech USA) on pellet quality. The 5 treatments consisted of a control, or the control with 2 levels of formic acid (3.6 g/kg and 6 g/kg), or the control with a blended product containing 600 g/kg formic acid and 400 g/kg lignosulfonate (blended product was included at 6 g/kg and 10 g/kg of the diet). Diets were steam conditioned (245 mm×1397 mm Wenger twin shaft pre-conditioner, Model 150) for approximately 30 sec and pelleted on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4.8 mm × 31.8 mm pellet die (L:D 6.7). The production rate was set at 900 kg/h. Treatments were pelleted at 3 separate time points to provide 3 replicates per treatment. Samples were collected directly after discharging from the pellet mill and cooled in an experimental counterflow cooler. Samples were then analyzed for pellet durability index using the Holmen NHP 100 (TekPro Ltd, Norfolk, UK) and via both standard and modified tumble box methods. Pellet hardness was determined by evaluating the peak amount of force applied before the first signs of fracture. Pellets were crushed perpendicular to their longitudinal axis using a texture analyzer (Model TA-XT2, Stable Micro Systems). Pellet samples were ground within replicate and mixed with distilled water. The samples were allowed to rest, the liquid was decanted into the electrode vessel and the pH was determined via potentiometer and electrode. Voltage and amperage data was collected via Supco DVCV Logger (Supco, New Jersey, US) and used to calculate kWh. Data were analyzed using the MIXED procedure in SAS 9.4, with pelleting run as the experimental unit. Increasing formic acid in the diet decreased (linear, $P < 0.001$) pH. No evidence for differences were observed for

pellet mill energy consumption, pellet durability index, or pellet hardness when adding formic acid or formic acid and lignosulfonate to the diet. In conclusion, neither pellet quality nor energy consumption was influenced by formic acid or lignosulfonate. However, as FA levels increased from 3.6 g/kg to 6.0 g/kg the pH levels decreased from 5.9 to 4.9 ($P < 0.001$)

Introduction

Organic acids have been included in swine feeds as a preservative agent to help control mold and bacteria growth and to help preserve overall feed and feed stuffs quality during storage. Organic acids, more specifically carboxylic acids, such as formic, fumaric, acetic, benzoic, propionic, and citric acid, are the most common organic acids. Organic acids have a general structure of R-COOH containing at least one or more carboxyl groups, and their infinity to give up their hydrogen electron defines them as either strong or weak acids. Most organic acids included in animal feeds are weak acids (Puyalto et al., 2018, Lückstädt C., 2007). These acids are used in feeds as a preservative agent to help control mold and bacteria growth in both feed and the GI tract (Mroczek et al., 2005, Argüello et al., 2013, Lynch et al., 2017). While it is one of the weakest organic acids, formic acid has one of the lowest minimum inhibitory concentration on Escherichia coli, and Salmonella Typhimurium (Lückstädt C., 2007). This provides a potential opportunity for organic acids, especially formic acid, to be used to decrease and limit antibiotic use. In addition to improving pig health, previous research demonstrated that formic acid improved growth rate and feed efficiency (Manzanilla et al. 2004, Ravindran and Kornegay, 1993). This is consistent with other research showing that organic acids increased the ATTD of CP, DM, fat, and GE (Hossain et al., 2018 Gerritsen et al., 2010, Upadhaya et al., 2018). These improvements in digestibility can be attributed to increasing gastric acidity which

can assist in gastric enzyme activation. Pigs go through an adjustment period post weaning and during this time they do not produce enough hydrochloric acid to lower the gastric pH in order to activate the gastric enzymes (Ravindran and Kornegay 1993). These potential improvements in nutrient digestibility and animal health could be correlated with improved pig performance. Researchers have however observed a decrease in average daily gain when organic acids were added to the diet (Nyguyen et al., 2019).

When feeding pelleted swine diets, the reduction of pellet quality is a concern as it can negatively affect swine feed efficacy (Nemechek et al., 2015, Stark et al., 1993). Although organic acids may improve pig performance, there is limited research on the influence of diet acidification on pellet quality. Villanueva et al. (2018) showed that a pH of 4.5 can influence gelatinization temperature depending on starch type. On rice starch, lowering pH decreased gelatinization temperature while it increased in the case of tapioca starch. As gelatinization can be correlated with pellet durability, it can be hypothesized that acidifying diets could drop pellet quality (Gilpin et al. 2002). Abdollahi et al. (2018) saw no markable change in pellet durability index when including an acidifier up to 10 g/kg. However, previous research has demonstrated the ability of pellet binders to improve pellet quality. Lignosulfonate is a by-product of the paper milling industry that has been used as a feed additive to improve pellet quality (Chang et al., 1977). Abadi et al. (2019) demonstrated improvements in pellet quality when adding up to 5 g/kg calcium lignosulfonate to swine diets. Others have reported similar effects with inclusions reaching up to 10 g/kg of lignosulfonate in the diets (Acar et al., 1991, Corey et al., 2014). Therefore, the objective of this study was to evaluate the effect of formic acid with and without lignosulfonate on pellet quality, feed pH, and pellet mill energy consumption.

Materials and Methods

This study was conducted at the Kansas State University O.H. Kruse Feed Technology and Innovation Center in accordance with Current Good Manufacturing Practices (CGMPs).

Diet Manufacturing

A total of 5 diets were pelleted to determine the effects of formic acid (FA) and the combination of FA and lignosulfonate (FA+LS) on diet pH, pellet quality, and pellet mill energy consumption. The 5 treatments (Table 1.1) consisted of a control, or the control with 2 concentrations of 95% formic acid (FA; 3.6 g/kg and 6 g/kg; Douda Diesel, Decatur, AL) or the control plus two concentrations (6.0 g/kg or 10 g/kg of a 600 g/kg FA and 400 g/kg lignosulfonate product (LS; 50% dry matter Lignotech USA, Rothschild WI)). This resulted in the 5 dietary treatments: Control, 3.6 g/kg FA, 6.0 g/kg FA, 6.0 g/kg FA+LS, and 10.0 g/kg FA+LS. Added concentrations of FA+LS blend were chosen to include the same concentrations of FA as the two FA treatments. Basal diets were mixed in a 1-ton Hayes and Stolz double ribbon mixer (Burleson, TX). Basal diets were equally divided to be mixed with the test articles before pelleting. The test articles were added and individually mixed directly, prior to pelleting using a Davis Paddle Mixer (Model S-3, Bonner Springs, KS). Pellets were produced from the mash diets via steam conditioning (245 mm×1397 mm length Model 150, Wegner twin staff pre-conditioner, Sabetha, KS) and subsequently pelleted using a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.8 mm × 31.8 mm pellet die (L:D 6. 7). The feeder rate was set at a constant rate to achieve approximately 900 kg/hr. The target conditioning temperature and time was held at 81-83°C for 30 seconds. The temperature was achieved by adjusting steam addition using 25-30 PSI steam pressure. Each treatment was pelleted in 3 separate 226.8 kg batches in order to provide 3

replicates per treatment. Time of processing served as a blocking factor and order of pelleting each treatment was randomized within each block.

Three pellet samples were collected from the cooler during each replication for analysis of pellet durability index and pellet hardness testing. Hot pellet temperature and production rate measurements were collected to correspond to each sample. Pellet mill energy consumption was measured using a Supco DVCV Logger (Supco, New Jersey, US).

Sample Analysis

Samples were tested for Pellet Durability Index (PDI) using the ASAE Standard S269.5 tumble box and modified tumble box methods (STD and MOD, respectively) and Holmen NHP100 (60sec, 70mbar) (TekPro Ltd, Norfolk, UK). For pellet hardness, 25 pellets between 10-12 mm in length were randomly chosen from the cooled pellet samples for each replicate to be analyzed. Pellets were crushed perpendicular to the longitudinal axis and the peak force (kg) required to cause the first fracture was recorded according to the methods described by Fahrenholz (2012). Pellet pH was determined by grinding 15 g of pellet samples for each replicate. Ground samples were placed in a flask and mixed with 100 ml of distilled water. The mixture was agitated until feed was suspended in the water and was free of clumps for 30 min. using a mechanical stirrer or shaker. The sample was allowed to rest for 10 minutes before the liquid was decanted into the electrode vessel and the pH was determined via potentiometer and electrodes (AACC Method 02-52.01). Moisture analysis was completed in duplicate for each mash, condition mash and cooled pellet sample. Aluminum pans were weighed, and a 2 g sample were placed in the drying oven at 105˚C for 24 hrs. Samples were weighed back for moisture calculation (100 – ((Dried sample weight / Initial sample weight) × 100))) (AOAC Method 934.01). An average of the 2 moistures was reported.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Experimental unit was pelleting run with a random effect of pelleting period. Results were considered significant if $P \leq 0.05$, and marginally significant if $P \leq 0.10$.

Results and Discussion

As the formic acid concentration increased from 3.6 g/kg to 6.0 g/kg, pH decreased from 5.9 to 4.9 ($P < 0.001$) (Table 1.3) regardless of lignosulfonate inclusion. There were no overall effects ($P > 0.05$) of dietary treatments on pellet mill energy consumption or hot pellet temperature (Table 1.2). Therefore, formic acid and lignosulfonate did not influence pellet quality. This was unexpected as Villanueva et al. (2018) showed that a pH of 4.5 can influence gelatinization temperature depending on starch type. On rice starch, lowering pH increased gelatinization temperature. However, when the pH of tapioca starch was decreased the gelatinization temperature decreased. As gelatinization can be correlated with pellet durability, it can be hypothesized that acidifying diets could potential decrease pellet quality (Gilpin et al. 2002). However, there were no evidence of difference ($P > 0.05$) between dietary treatment effects on pellet durability index for the standard tumble box, modified tumble box, Holman 100 or pellet hardness (Table 1.3). Therefore, the data reported herein indicates that formic acid may be included in the diet without negatively impacting pellet quality. More research is needed to evaluate the effects of other organic acids and the salts of those acids to evaluate their effects on pellet quality. As lignosulfonate was included in the diet up to 4.0 g/kg there were no differences ($P > 0.05$) (Table 1.3) in PDI regardless of PDI testing method used. This was unexpected as research completed by Abadi et al. (2019) observed between a 2% and 10% increase in PDI in

diets containing soy oil, but not chicken fat powder, when calcium lignosulfonate was added to the diet. However, when the soy oil was 3% and calcium lignosulfonate levels were 1%, PDI decreased 20% as compared to feed without added fat or calcium lignosulfonate, when using a Holmen pellet tester. It is hypothesized that the concentration of lignosulfonate was too low in treatment diets used herein to elicit a positive response in pellet durability index. Future research needs to be considered to determine if formic acid may offset the effect of the lignosulfonate on pellet quality.

Conclusion

In conclusion, the addition of formic acid, with and without lignosulfonate, had no effect on pellet quality or pellet mill performance. There was a decrease in pH as formic acid inclusion increased.

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Tables

Table 1-1: Diet composition, as-is basis.

Ingredients, g/kg	Control	3.6 g/kg FA[1]	6.0 g/kg FA	6.0 g/kg FA+LS[2]	10 g/kg FA+LS
Ground Corn	661.8	658.2	655.8	655.8	651.8
SBM, Dehulled 46.5%	282.5	282.5	282.5	282.5	282.5
Soy Oil	15.0	15.0	15.0	15.0	15.0
L-Lysine - HCL	5.5	5.5	5.5	5.5	5.5
DL- Methionine	2.1	2.1	2.1	2.1	2.1
L- Threonine	2.3	2.3	2.3	2.3	2.3
L-Tryptophan	0.5	0.5	0.5	0.5	0.5
L-Valine	1.6	1.6	1.6	1.6	1.6
Monocalcium Phosphate 21%	11.0	11.0	11.0	11.0	11.0
Limestone	7.5	7.5	7.5	7.5	7.5
Salt	6.0	6.0	6.0	6.0	6.0
Trace mineral premix[3]	1.5	1.5	1.5	1.5	1.5
Vit premix[4]	2.5	2.5	2.5	2.5	2.5
Phytase[5]	0.3	0.3	0.3	0.3	0.3
Formic acid[6]	-	3.6	6.0	-	-
Blend	-	-	-	6.0	10.0
Total, g	1000.00	1000.00	1000.00	1000.00	1000.00

[1] Formic Acid (Douda Diesel, Decatur, AL)

[2] Fa+LS contained 60% Formic Acid 40% Lignosulfonate.

[3] Provided 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 per kg of premix.

[4] Provided 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 per kg of premix.

[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] Formic acid and formic acid+lignosulfonate were added once basal batches were split directly prior to the pelleting period.

Table 1-2:Effect of Formic acid and Lignosulfonate on pellet mill performance.[1,2]

Item	Control	3.6g/kg FA[3]	6.0g/kg FA	6.0g/kg FA+LS[4]	10g/kg FA+LS	SEM	P-Value
Production Rate, kg/hr	932.4	924.8	926.4	935.7	938.4	-	-
Conditioning Temperature, ˚C	82.6	82.4	82.6	82.4	82.4	0.13	0.167
Hot pellet temperature, ˚C	86.4	86.5	86.6	86.3	86.5	0.35	0.361
kWh/T	13.1	13.0	12.8	13.1	12.8	0.24	0.826

[1] Corn, Soybean meal-based nursery pig diets were steam pelleted for approximately 30 s at 82˚C targeted conditioning temperature with a production rate target of 900 kg/hr. on a 30-horsepower pellet mill with a 4.8 mm × 31.8 mm pellet die (L:D 6.6).

[2] Diets were pelleted at 3 separate time points to achieve 3 replicated per treatment

[3]Formic Acid (Douda Diesel, Decateur AL)

[4]Fa+LS contained 600g/kg Formic Acid 400g/kg Lignosulfonate.

[5] Kilowatt hour per ton

Table 1-3: Effect of formic acid and lignosulfonate on pellet quality and feed pH. [1,2]

Item,[3,4]	Control	3.6g/kg FA[5]	6.0g/kg FA	6.0g/kg FA+LS[6]	10g/kg FA+LS	SEM	*P*-Value
Holman 100 with Filter, %[7]	88.0	89.3	89.6	89.0	88.7	1.69	0.947
Standard tumble box, %[8]	85.3	86.9	86.4	87.2	86.3	1.34	0.786
Modified tumble box, %[9]	75.2	77.9	76.9	78.7	77.7	2.23	0.762
Pellet hardness, kg10	6.57	6.59	6.28	6.81	6.70	0.33	0.813
Moisture, %	8.32	8.29	8.63	8.59	8.70	0.22	0.289
pH	5.9[a]	5.1[b]	4.9[c]	5.3[b]	4.9[c]	0.06	0.001

[1] A corn soybean meal-based nursery pig diets were steam pelleted for approximately 30 s at 82˚C targeted conditioning temperature with a production rate target of 900 kg/hr. on a 30-horsepower pellet mill with a 4.8 mm × 31.8 mm pellet die (L:D 6.67).

[2] Diets were pelleted at 3 separate time points to achieve 3 replicates per treatment.

[3] All PDI analysis and pH analysis was completed in duplicate on the 3 samples collected for each treatment per replication.

[4] Superscripts (a,b,c) denote significant differences ($P < 0.05$).

[5] Formic Acid (Douda Diesel, Decatur, AL).

[6] FA+LS contained 600g/kg Formic Acid 400g/kg Lignosulfonate.

[7] Holmen NHP Pellet Tester 100 ran for 60 seconds at 70 mbar with a Holmen Filter. A No. 5 Screen was used to sift pellets.

[8] KSU Standard Tumble Box ran for 600 seconds with no added abrasive. A No. 5 Screen was used to sift pellets.

[9] KSU Modified Tumble Box ran for 600 seconds with three ¾ -inch hex nuts as the abrasive. A No. 5 Screen was used to sift pellets.

[10] 25 pellets between 0.39 and 0.47 in length were chosen at random, pellets were crushed perpendicular to the longitudinal axis and the peak force (kg) was recorded.

Chapter 2 - Efficacy of Medium Chain Fatty Acids and Commercially Available Fatty Acid-Based Feed Additives as a Mitigation Strategy Against Porcine Epidemic Diarrhea Virus and Porcine Reproductive and Respiratory Virus

Abstract

Research has demonstrated that swine viruses can be transmitted via feed. Therefore, strategies are needed to prevent or mitigate swine viruses in feed. Chemical feed additives are one such strategy that has been shown to have potential utility for this purpose. Therefore, the objective of this study was to evaluate the efficacy of a commercially available formaldehyde-based feed additive, medium chain fatty acid blend (MCFA), and commercially available fatty acid-based products for mitigation of porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) as viral mitigants in a feed matrix. Experimental treatments consisted of: 1) non-treated, individually inoculated virus controls (positive control); 2) 0.33% commercial formaldehyde-based product (Sal Curb; Kemin Industries, Inc.; Des Moines, IA); 3) 0.50% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO); 4) 0.25%; 5) 0.50%; or 6) 1.00% of commercial dry mono and diglyceride-based product (Furst Strike; Furst-McNess Company, Freeport, IL); 7) 0.25%; 8) 0.50%; or 9) 1.00% of commercial dry mono and diglyceride-based product (Furst Protect; Furst-McNess Company, Freeport, IL); 10) 0.25%; 11) 0.50%; or 12) 1.00% dry mono and diglyceride-based experimental product (Furst-McNess Company, Freeport, IL). In total, there were 12 treatments with 3 replications per treatment. A complete swine feed was treated with each chemical treatment before inoculation

with 10^6 $TCID_{50}$/g of feed with PEDV or PRRSV. Post inoculation feed was held at ambient temperature for 24 h before being analyzed via qRT-PCR. The analyzed values represent the cycle threshold (Ct). A lower Ct value indicates a greater concentration of detectable viral nucleic acid. Formaldehyde and MCFA decreased ($P < 0.05$) the detectable RNA concentration of PEDV and PRRSV compared to all other treatments. Furst Strike, Furst Protect, and the experimental product did not significantly reduce detectable concentrations of RNA for PEDV or PRRSV. In conclusion, MCFA and formaldehyde chemical treatments are effective at reducing nucleic acid concentrations of PEDV and PRRSV in feed.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first seen in the United States in the late 1980s. The virus causes reproductive losses due to early farrowing, late term abortions, and dead or mummified litters, along with pneumonia and reduced growth performance. In older pigs, it can cause respiratory distress including pneumonia, fever, and stunting due to disease. The economic impact of PRRSv is estimated to be at least 600 million dollars annually (Holtkamp et.al 2013). Porcine epidemic diarrhea virus (PEDV) was introduced into the United States in 2013 and became prevalent throughout 2014 reaching 29 states by that May. The virus causes 80-100% mortality in neonatal piglets and high morbidity in pigs of other ages (Schulz and Tonsor, 2015). With concerns about virus transmission onto farms, research has been conducted to evaluate possible transmission routes. One such route was feed coming onto the farm. It has been shown that PEDv can be transmitted through feed in controlled studies (Dee et al., 2014a; Pasick et al., 2014) and by epidemiology research (Aubry et al., 2017). The contamination of feed can be caused by ingredients, transportation, manufacturing surfaces, and during feed storage through dust and fecal matter. Key points of viral entry into the feed mill are

from feed ingredients, worker and visitor foot traffic, and receiving or delivery trucks (Cochrane et al. 2016, Schumacher et al. 2017). Knowing that feed is a vector for disease and that it can easily be introduced into a feed mill environment, there is great interest in determining the best method to reduce the risk of viral transmission through feed. One option is the use of different feed chemical mitigants and commercial feed additives to reduce viral load in feed. Previous research has evaluated the effects of medium chain fatty acids (MCFA), essential oils, organic acids, formaldehyde, and their combinations as viral mitigants in feed (Cochrane 2015, Dee et al 2020, Gebhardt et al. 2018). Research has demonstrated the efficacy of MCFA and formaldehyde to significantly reduced PEDV RNA levels in swine feed (Gebhardt et al. 2020, Lerner et al. 2020). The bulk of the previous research has been focused on feed inoculated with PEDV. However, Dee and others (2020) have demonstrated that PRRSv can survive in a feed matrix and infect pigs. The same study also showed that much like PEDv, PRRSv is susceptible to mitigants such as MCFAs and formaldehyde. Along with testing these proven products, questions arise about other products and fatty acids and their efficacy as viral mitigants in feed (Dee et al. 2020). Therefore, the objective of this study was to evaluate the efficacy of commercial formaldehyde, MCFA, and commercially available fatty acid-based products against PEDV and PRRSV as viral mitigants in a feed matrix.

Materials and Methods

A complete corn and soybean meal-based swine gestation diet (Table 2.1) was utilized—it did not contain specialty ingredients (whey, specialty soybean meal, animal plasma protein, or fish products) or antibiotics. All feed samples tested negative for PEDV and PRRSV by quantitative real time reverse transcription PCR (qRT-PCR) prior to chemical treatment.

Chemical treatments

Chemical treatments included were 1) Non-Treated, inoculated Control (Positive Control); 2) 0.33% of the liquid commercial formaldehyde-based product (Sal Curb, Kemin Industries, Des Moines IA); 2) 0.33% commercial formaldehyde-based product (Sal Curb; Kemin Industries, Inc.; Des Moines, IA); 3) 0.50% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO); 4) 0.25%; 5) 0.50%; or 6) 1.00% of commercial dry mono and diglyceride-based product (Furst Strike; Furst-McNess Company, Freeport, IL); 7) 0.25%; 8) 0.50%; or 9) 1.00% of commercial dry mono and diglyceride-based product (Furst Protect; Furst-McNess Company, Freeport, IL); 10) 0.25%; 11) 0.50%; or 12) 1.00% dry mono and diglyceride-based experimental product (Furst-McNess Company, Freeport, IL). In total, there were 12 treatments with 3 replications per treatment.

Feed Mixing and Mitigant Addition

Chemical treatments were added to 100g batches of feed and mixed for 15 minutes in a mason jar mixer (Central Machine Shop, Purdue University, West Lafayette, IN) with 10-7.9 mm hex nuts for agitation. 22.5g of treated feed was placed into three separate polyethylene bottles (250 mL Nalgene bottle, square wide-mouth high-density polyethylene; Thermo Fisher Scientific, Waltham, MA) to achieve 3 replicates per treatment. Polyethylene bottles were stored at ambient temperature for 24 h before inoculation.

Virus Isolate and Inoculation

The samples were inoculated in the polyethylene container at the Kansas State University Veterinary Diagnostic Laboratory with either the PEDV USA/Co/2013 (KF272920.1) or the PRRSV 1-7-4. Both PEDV and PRRSV were provided by the Animal Disease Research and Diagnostic Laboratory at South Dakota State University. Each viral inoculum contained an initial

infectious titer of 10^7 $TCID_{50}$/mL. All treatments were inoculated by pipetting 2.5 mL of each viral inoculum individually into each bottle, resulting in a final viral concentration of 10^6 $TCID_{50}$/g of feed. Bottles were then shaken for 15s to distribute each virus throughout the feed matrix.

Real Time PCR Analysis

For 24 h post inoculation, bottles were held at ambient temperature. Then, 100 mL of phosphate buffered saline (PBS; pH 7.2 1X, Life Technologies, Grand Island, NY) was added to each inoculated bottle and shaken to ensure even mixing. Bottles were placed in a refrigerator at 4 °C for 24 h to allow feed to settle. Quantitative real time reverse transcription PCR was conducted using methods previously described from Gebhardt et al. (2018). Supernatant was collected and placed into a 96 well plate for qRT-PCR. Supernatant from the 96 well plate was 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 indicate cycle threshold at which virus was detected.

Statistical Analysis

Each 250 mL bottle was considered as an experimental unit resulting in 3 replicates per treatment. For inoculated samples containing no detectable genetic material following 45 cycles, a value of 45.0 was assumed for samples with no detectable RNA for analysis. Data were analyzed using the PROC GLIMMAX procedure in SAS (SAS Institute 9.4, Inc. Cary, NC). Results were considered significant if $P \leq 0.05$ and marginally significant if $P \leq 0.10$.

Results and Discussion

PEDv was detected in the positive control treatment with a Ct value of 31.2 (Table 2.2), and there was a significant ($P = 0.005$) overall treatment effect. Both formaldehyde and the MCFA blend resulted in increased ($P < 0.05$) Ct values for PEDv of 3 and 2.3 respectively. Similarly, previous research using formaldehyde and MCFA blends as mitigants has demonstrated that they are both effective as reducing detectable PEDv RNA in feed (Cochrane et al. 2020, Gebhardt et al. 2020, and Lerner et al. 2020). Dee and others (2020) however, did not see a numerical decrease in Ct values in feed when treated with formaldehyde or products containing MCFAs. They did observe a decrease in infectivity of pigs fed either products when testing rectal swabs. This could be a result of using a Swiffer dry mop to sample the feed pan. Research has shown that using a dry mop to sample feed surfaces for PEDv do not result in Ct values as low as testing a feed ingredient (Stewart, 2020). Furst Protect, Furst Strike, and the Dry Prototype did not increase PEDv ($P > 0.05$) Ct values over the positive control. For Furst Protect, this is similar to results seen by Dee et.al. (2020) in feed. Notably, they did see a decrease in the infectivity in the in vivo model when evaluating fecal samples. This raises the question as to the efficacy of dry products in an in-vitro model. Previous research by Cochrane and others (2020) showed that liquid lauric acid significantly reduced PEDv Ct values unlike a dry commercial lauric acid product in an in-vitro model. Similarly, Muckey and others (2016) saw that when sanitizing feed mill surfaces, dry products were less effective than liquid products at reducing detectable PEDv RNA.

PRRSv was detected in the positive control treatment with a Ct value of 30.0, and there was an overall treatment effect ($P < 0.001$). Formaldehyde resulted in the greatest increase ($P < 0.05$) in Ct values for PRRSv as compared to all other treatments, with 2 of the 3 samples

returning no detectable RNA virus. The MCFA blend was intermediate ($P < 0.05$) but still significantly increased ($P < 0.05$) Ct values compared to the 3 dry products and the positive control. This is similar to what Dee et.al. (2020) saw when using SalCurb and MCFAs based products, as they saw mixed numerical results for feed Ct values. However, much like in PEDv, they did see a decrease in PRRS positive pigs testing serum post-mortem for pigs fed SalCurb and MCFA products. This difference could still be an effect of sampling method. In this trial Furst Protect, Furst Strike, and the Dry Prototype did not increase ($P>0.05$) Ct values over the positive control. For Furst Protect, this is again similar to results seen by Dee et.al. (2020) in feed. They did however see decrease the infectivity in the pig, which continues to raise concern about the efficacy of dry products in an in-vitro model.

Conclusion

In conclusion, formaldehyde and the MCFA blend both reduced the detectable RNA of either PEDV or PRRSv as expected. Furst Protect, Furst Strike, and Prototype A did not reduce the detectable RNA of either PEDV or PRRSv. This is similar to what was seen in other trials when using Furst Protect in feed. While there was no effect in feed Ct values, Furst Protect did reduce infectivity when looking at both viruses. This data raises the question of whether the physical product form of a product (dry vs. wet) influences mitigant efficacy when studied in an in-vitro model. Future research is needed to validate an in-vitro model for dry mitigants in feed.

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Tables

Table 2-1:Diet composition, as-fed basis.

Ingredient, %	
Corn	78.40
Soybean meal, 46.5%	17.27
Soy oil	0.50
Monocalcium phosphate 21%	1.30
Limestone	1.30
Salt	0.50
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Sow add pack[3]	0.25
Phytase[4]	0.08
Total	100

[1] 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.

[2] 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.

[3]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.

[4] 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

Table 2-2: Efficacy of chemical mitigants used to treat swine feed on PEDV and PRRSV detection using qRT-PCR. [1]

Product:	Positive Control[2]	Liquid Formaldehyde[3]	Liquid MCFA[4]	Dry Furst Protect			Dry Furst Strike			Dry Prototype A			SEM	P-Value
Concentration:	N/A	0.325%	0.50%	0.25%	0.50%	1.0%	0.25%	0.50%	1.0%	0.25%	0.50%	1.0%		
PEDV														
qRT-PCR Ct[5]	31.2^{b}	34.2^{a}	33.5^{a}	31.3^{b}	30.5^{b}	31.4^{b}	31.5^{b}	30.8^{b}	31.7^{b}	31.1^{b}	30.7^{b}	31.0^{b}	0.608	*0.005*
PRRSV														
qRT-PCR Ct	30.0^{c}	$42.0^{a\ (2/3)}$	34.2^{b}	30.2^{c}	30.3^{c}	30.6^{c}	29.6^{c}	30.0^{c}	30.3^{c}	30.5^{c}	30.4^{c}	31.2^{c}	0.975	*0.0001*

[1] An initial tissue culture (2.5 mL of diluted PEDV or PRRSV, 10^7 $TCID_{50}$/mL) was inoculated into 22.5 g of swine feed. Samples were stored for 24 h post-inoculation, then 100 mL of PBS was added, samples then sat overnight in fridge before being pulled for PCR analysis.

[2] Positive control: non-chemically treated feed inoculated with virus.

[3] Sal CURB (Kemin Industries, Des Moines, IA) was included at the label dosage levels 3.25 g/kg

[4] MCFA treatment consisted of a 1:1:1 blend of C6:C8:C10 (hexanoic, octanoic, and decanoic acids, respectively) (Sigma Aldrich, St. Louis, MO)

[5] Cycle threshold (Ct) required to detect viral nucleic acid. A high Ct value indicates less viral nucleic acid present.

[a,b] Means with differing superscripts differ $P < 0.05$ within row.

[(x/x)] Superscripts denote number of samples containing no detectable genetic material following 45 cycles. A value of 45.0 was assumed for samples with no detectable RNA for analysis.

MCFA = medium chain fatty acid, PEDV = Porcine Epidemic Diarrhea Virus, PRRSV = Porcine Reproductive and Respiratory Syndrome Virus, qRT-PCR = quantitative real time reverse transcription polymerase chain reaction

Chapter 3 - Evaluation of Feed Mitigant Efficacy for Control of Porcine Epidemic Diarrhea Virus and Porcine Reproductive and Respiratory Syndrome Virus when Inoculated Either Alone or Co-Inoculation

Abstract

Research has demonstrated that swine feed can be a fomite for viral transmission and certain feed additives can effectively reduce viral contamination. However, additional information is needed to evaluate the efficacy of feed additives when inoculated with more than one virus. Therefore, the objective of this study was to evaluate the efficacy of two feed additives for mitigation of porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) individually or when co-inoculated. Feed additives included: 1) no treatment; 2) 0.33% commercial formaldehyde-based product (Sal Curb, Kemin Industries, Des Moines, IA); and 3) 0.50% medium chain fatty acids blend (MCFA; 1:1:1 ratio of C6:C8:C10, Sigma Aldrich, St. Louis, MO). Samples were inoculated with PEDV alone, PRRSV alone, or combination of PEDV and PRRSV at an inoculation concentration of 10^6 $TCID_{50}$/g for all viruses. Once inoculated, feed was stored at ambient temperature for 24-h before analyzed via qRT-PCR. For samples inoculated with PEDV or PRRSV alone, a qRT-PCR assay was used which was designed to detect PEDV or PRRSV nucleic acid. For co-inoculated samples, an assay was designed to independently detect both PEDV and PRRSV within a single reaction. For PEDV alone, there was marginally significant evidence that feed additive resulted in differences

in cycle threshold (Ct) value ($P = 0.052$), but no evidence for pairwise differences, using a Tukey multiple comparison adjustment, was observed. For PRRSV alone, formaldehyde effectively increased Ct compared to the untreated control and MCFA treatment ($P < 0.05$). For co-infection of PRRSV and PEDV, MCFA and formaldehyde effectively increased Ct ($P < 0.05$) in comparison to non-treated feed. In summary, formaldehyde effectively increased Ct values in feed when contaminated with PRRSV, while both mitigants effectively increased Ct value in feed when co-inoculated with PRRSV and PEDV.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first seen in the United States in the late 1980s. The virus causes reproductive losses including early farrowing, late term abortions, and dead or mummified litters, along with pneumonia and reduced growth performance. In older pigs, it can cause respiratory distress including pneumonia, fever, and stunting due to disease. The economic impact of PRRSV is estimated to be at least 600 million dollars annually (Holtkamp et.al 2013). Porcine epidemic diarrhea virus (PEDV) was introduced into the United States in 2013 and became prevalent throughout 2014. The virus causes 80-100% mortality in neonatal piglets and high morbidity in pigs of other ages (Schulz and Tonsor 2015). With concerns about virus transmission onto farms, research has been done to evaluate possible transmission routes. One potential route was feed delivery to the farm. It has been shown that PEDV can be transmitted through feed in controlled studies (Dee et al., 2014a; Pasick et al., 2014) and by epidemiology research (Aubry et al., 2017). The contamination of feed can be caused by ingredients, transportation, manufacturing surfaces, and dust and fecal matter contamination during feed storage. Key points of viral entry into the feed mill are from feed ingredients, worker and visitor foot traffic, or receiving or delivery trucks (Cochrane et al. 2016,

Schumacher et al. 2017). Knowing that feed is a vector for disease and that it can easily be introduced into a feed mill environment there is great interest in determining the best method to reduce the risk of viral transmission through feed. One option is the use of different feed chemical mitigants and commercial feed additives to reduce viral load in feed. Previous research has evaluated the effects of medium chain fatty acids (MCFA), essential oils, organic acids, formaldehyde, and their combinations as viral mitigants in feed (Cochrane 2015, Dee et al., 2020, Gebhardt et al. 2018). Research has demonstrated the efficacy of MCFA and formaldehyde to significantly reduced PEDV RNA levels in swine feed (Gebhardt et al., 2020, Lerner et al., 2020). The bulk of the previous research has been focused on feed inoculated with PEDV only. However, Dee et al. (2020) have demonstrated that PRRSV can also survive in a feed matrix and infect pigs. The authors also demonstrated that feed can be inoculated with multiple swine viruses simultaneously and both infect pigs. The viruses can also be susceptible to mitigants as confirmed by PCR analysis. Currently, as most studies have evaluated only one swine virus in feed at a time, the bulk of PCR analysis has been done as a simplex test only looking to the Ct values for the target virus. The development of duplex PCR test has been done before to detect varying porcine pathogens such as Brachyspira hyodysenteriae and Brachyspira pilosicoli (La, Phillips et al., 2003) or porcine circovirus 2 and porcine circovirus 3 (Cheng Yahn et al., 2018). Therefore, the objective of this study was to evaluate the efficacy of two feed additives for mitigation of porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) individually or when co-inoculated.

Materials and Methods

A complete corn and soybean meal-based swine gestation diet (Table 3.1) was utilized—it did not contain specialty ingredients (whey, specialty soybean meal, animal plasma protein, or

fish products) or antibiotics. All feed samples tested negative for PEDV and PRRSV by quantitative real time reverse transcription PCR (qRT-PCR).

Chemical Treatments

Three separate experiments were conducted to test the efficacy of two feed additives. Samples were inoculated with: 1) PEDV alone; 2) PRRSV alone; 3) combination of PEDV and PRRSV. Samples had an inoculation concentration of 10^6 $TCID_{50}$/g for all viruses. Feed additives included: 1) no treatment; 2) 0.33% commercial formaldehyde-based product (Sal Curb, Kemin Industries, Des Moines, IA); and 3) 0.50% medium chain fatty acids blend (MCFA; 1:1:1 ratio of C6:C8:C10, Sigma Aldrich, St. Louis, MO).

Feed Mixing and Mitigant Addition

Chemical treatments were added to 100g batches of feed and mixed for 15 minutes in a mason jar mixer (Central Machine Shop, Purdue University, West Lafayette, IN) with 10-7.9 mm hex nuts for agitation. For treatments consisting of 1 virus inoculate, 22.5 g of treated feed was placed into three separate polyethylene bottles (250 mL Nalgene bottle, square wide-mouth high-density polyethylene; Thermo Fisher Scientific, Waltham, MA) to achieve 3 replicates per treatment. For treatments containing the 2 virus inoculates, 20.0 g of feed was placed into three separate 250 mL polyethylene bottles to ensure equal virus titer levels for the individual virus across all treatments. Polyethylene bottles were stored at ambient temperature for 24 h before inoculation.

Virus Isolate and Inoculation

The samples were inoculated in the polyethylene container at the Kansas State University Veterinary Diagnostic Laboratory with either the PEDV USA/Co/2013 (KF272920.1) or the PRRSV 1-7-4. Both PEDV and PRRSV were provided by the Animal Disease Research and

Diagnostic Laboratory at South Dakota State University. Each viral inoculum contained an initial infectious titer of 10^7 $TCID_{50}$/mL. All treatments were inoculated by pipetting 2.5 mL of each viral inoculum into each bottle as required for the treatment, resulting in a final viral concentration of 10^6 $TCID_{50}$/g of feed. Bottles were then shaken for 15 s to distribute each virus throughout the feed matrix.

Real Time PCR Analysis

For 24 hours post inoculation bottles were held at ambient temperature. Then, 100 mL of phosphate buffered saline (PBS; pH 7.2 1X, Life Technologies, Grand Island, NY) was added to each inoculated bottle and shaken to ensure even mixing. Bottles were placed in a refrigerator at 4 °C for 24 h to allow feed to settle. Quantitative real time reverse transcription PCR was conducted using methods previously described from Gebhardt et al. (2018). Supernatant was collected and placed into a 96 well plate for qRT-PCR. Supernatant from the 96 well plate was 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. Samples inoculated with PEDV or PRRSV alone were analyzed using a simplex PCR test. Co-inoculated samples were analyzed using a duplex PCR test. One negative extraction control consisting of all reagents except the sample will be included in each extraction. The extracted RNA was frozen at −20 °C until assayed by qRT-PCR. Analyzed values indicate cycle threshold at which virus was detected.

Statistical Analysis

Each 250 mL bottle was considered as an experimental unit resulting in 3 replicates per treatment. For inoculated samples containing no detectable genetic material following 45 cycles,

a value of 45.0 was assumed for samples with no detectable RNA for analysis. Data was analyzed separately for inoculations of PEDV alone, PRRSV alone, or combination of PEDV and PRRSV. Data were analyzed using the PROC GLIMMAX procedure in SAS (SAS Institute 9.4, Inc. Cary, NC), with main effects of inoculation type and mitigant. Results were considered significant if $P \leq 0.05$ and marginally significant if $P \leq 0.10$.

Results and Discussion

For samples inoculated with PEDV or PRRSV alone, a simplex qRT-PCR assay was used which was designed to detect PEDV or PRRSV nucleic acid. For co-inoculated samples, a duplex assay was designed to independently detect both PEDV and PRRSV within a single reaction. For PEDV alone, there was marginally significant evidence that feed additive resulted in differences in cycle threshold (Ct) value ($P = 0.052$, Table 3.2), but no evidence for pairwise differences using a Tukey multiple comparison adjustment was observed. For PRRSV alone, formaldehyde effectively increased Ct compared to the untreated control and MCFA treatment ($P < 0.05$). For co-infection of PRRSV and PEDV, MCFA and formaldehyde effectively increased Ct ($P < 0.05$) in comparison to non-treated feed.

The data reported herein did not statistically detect a difference in PEDV Ct value in feed treated with formaldehyde or MCFA. However, formaldehyde and MCFA increased the PEDV Ct value in co-inoculated feed numerically. Previous research however demonstrated that formaldehyde and MCFAs can reduce PEDV Ct values by 2-5 when feed was inoculated with PEDV alone (Cochrane et al. 2020 Gebhardt et al. 2020, Lerner et al. 2020). Dee et al. (2020) however did not see a numerical increase in Ct values in feed inoculated with PEDV, PRRSV, and Seneca Valley Virus, when treated with formaldehyde or products containing MCFA. Notably however, they did see a decrease in infectivity of pigs fed either products when testing

rectal swabs via PCR (Dee et al., 2020). The authors reported that a Swiffer® dry mop was used to swab the feed pans and the feeders. The dry mops were then immersed in saline solution and agitated to release the collected feed sample. This sample collection method could potentially lead to the difference in reported results. Previous research has shown that using a dry mop to sample feed surfaces for PEDV do not result in Ct values as low as testing a feed ingredient for a virus. This is possibly due to a static charge that could help retain virus on the dry mop (Stewart, 2020).

Dee et al. (2020) demonstrated that PRRSV could survive in feed and furthermore infect pigs with the inoculated feed, when returning Ct values in feed between 24 and 34. When adding formaldehyde and MCFA based products, a numerical result in feed Ct values were mixed with some treatments reducing Ct values and others increasing them 6 days' post infection. When pulling samples at 15 days post infection they saw zero positive samples for feed treated with Formaldehyde or MCFA based products. When analyzing another MCFA based product they saw a complete reduction in PRRSV detection in feed tested at either 6- or 15-days post infection. However, much like in PEDV they did see a decrease in PRRSV positive pigs when Sal CURB or MCFA based products were added to the diet. The trial herein would show that both Sal CURB and MCFA based products decrease the amount of detectable PRRSV RNA in feed. This in feed difference could be the result of testing the complete feed vs a dry mop swab of the feed.

Conclusion

In conclusion, there was not a statistically significant change in PEDV Ct values of feed treated with formaldehyde or MCFA. In feed inoculated with PRRSV alone formaldehyde significantly increased the Ct values over untreated feed and feed treated with MCFA. For co-

inoculated feed both formaldehyde and MCFA increased PEDV and PRRSV Ct values. Overall, formaldehyde and MCFAs increased feed Ct values in co-inoculated feed and formaldehyde increased PRRSV Ct values against PRRSV alone.

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Tables

Table 3-1: Diet composition, as-fed basis.

Ingredient	%
Corn	78.40
Soybean meal, 46.5%	17.27
Soy oil	0.50
Monocalcium phosphate 21%	1.30
Limestone	1.30
Salt	0.50
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Sow add pack[3]	0.25
Phytase[4]	0.08
Total	100

[1] Provided 1.36 g Cu from copper sulfate; 72.72 mg Ca from calcium iodate; 14.09 mg Fe from ferrous sulfate; 1.36 g Mn from manganese sulfate; 54.54 mg Se from sodium selenite; and 14.09 g Zn from zinc sulfate per lb of premix.

[4] Provided 750,000 IU vitamin A from vitamin A acetate; 300,000 IU vitamin D from vitamin D3; 8,000 IU vitamin E from dl-α-tocopherol acetate; 600 mg menadione from menadione nicotinamide bisulfite; 6 mg B12 from cyanocobalamin; 9000 mg niacin from niacinamide; 5000 pantothenic acid from d-calcium pantothenate; and 1,500 mg riboflavin from crystalline riboflavin per lb of premix.

[5]Provided 0.035 g chromium, 750,000 IU vitamin A from vitamin A acetate; 4,000 IU vitamin E from dl-α-tocopherol acetate; 40 mg biotin, 400 mg folic acid, 180 mg pyridoxine, 100,000 mg choline, and 9,000 mg carnitine per lb of add pack.

[6] 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.

Table 3-2: Cycle threshold values for porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) when inoculated either alone or co-inoculation following application of feed additives. [1]

		Mitigant				
Virus:	Assay:	Untreated	MCFA[2]	Formaldehyde[3]	SEM	*P* =
PEDV	PEDV	31.2	33.5	34.2	0.69	0.052
PRRSV	PRRSv	30.0[b]	34.2[b]	42.0[a]	1.78	0.009
Co-inoculated						
PEDV quantification	Duplex	30.8[b]	31.9[a]	32.5[a]	0.23	0.006
PRRSV quantification	Duplex	30.0[b]	33.8[a]	34.7[a]	0.85	0.019

[1] Swine feed samples were inoculated with 10^6 $TCID_{50}$/g of PEDV, PRRSV, or PEDV and PRRSV co-inoculation, then analyzed using one of three quantitative real time reverse transcription polymerase chain reaction assays including an assay detecting PEDV only, an assay detecting PRRSV only, or an assay detecting and independently reporting quantification of both PEDV and PRRSV (duplex).

[2] Medium chain fatty acid blend (MCFA; 1:1:1 ratio of C6:C8:C10; Sigma Aldrich, St. Louis, MO) at 0.50% inclusion.

[3] Commercial formaldehyde-based feed additive (Sal CURB; Kemin Industries, Des Moines, IA) at 0.325% inclusion.

[a,b,c] Means within virus lacking common superscripts differ $P < 0.05$.

Chapter 4 - Decreasing Corn Particle Size Increases Energy and Nitrogen Digestibility in Gestating Sows

Abstract

Previous research has demonstrated that reducing the particle size of corn improved metabolizable energy utilization in many phases of swine production. One phase that has limited research thus far is gestation sows. The objective of this experiment was to determine the effects of corn particle size on the digestibility of protein (CP), and digestible energy (DE), metabolizable energy (ME), and nitrogen adjusted metabolizable energy (AMEn) in gestating sow diets. A total of 27 sows during the second phase of gestation (Day 40 to 74) were chosen and fed a common diet with one of 3 target average particle sizes (dgw) of corn ground to either 400, 800, or 1200 µm. Corn was ground using a 3 high roller mill (RMS model 924). Titanium dioxide (0.25%) was included in the diet as an indigestible marker for index digestibility calculations. Sows were fed experimental diets for 7 days to allow for diet adaptation before a 2-day collection period. At the beginning of the collection period, sows were fitted with a urinary catheter and urine was collected in buckets containing 20 mL of sulfuric acid. Fecal grab samples were also collected for each sow during the collection period. Subsamples were taken, mixed, analyzed for nitrogen, gross energy, and titanium levels to determine digestibility of protein and energy. Apparent total tract digestibility (ATTD) of crude protein (CP), coefficients for DE and ME, and DE, ME, AMEn and Corn ME content increased (linear, $P < 0.001$) as corn particle size was reduced from 1200-400 µm. The AMEn (88.5% DM) of the diet increased by 179 kcal/kg, which resulted in a 22.4 kcal/ kg improvement for every 100 µm decrease in particle

size. Calculated corn ME (88.5% DM) also increased by 28.5 kcal/kg for every 100 μm decrease in corn particle size.

Introduction

Feed costs represent approximately 70% of the cost of pork production (Patience et al., 2015). Therefore, swine producers strive to reduce the cost of feed by improving performance and nutrient efficiency. One way to achieve this goal is to improve nutrient digestibility through reducing corn particle size. Particle size reduction of grains continues to be used in the swine industry to increase nutrient utilization regardless of production phase (Lancheros et al., 2020). Both roller mills and hammermills are utilized to grind grains. The type of mill used is commonly chosen by capacity, target particle size, and energy efficiency (Hancock and Behnke, 2001). Roller mills are more efficient than hammermills but cannot grind as fine of particle size as a hammer mill. However, they do result in a more consistent particle size, which can help improve flowability (Gebhardt et al., 2018, Goodband et al., 2006). Hammermills, while less efficient, are also able to handle more fibrous feedstuffs while also being able to process cereal grains (Rojas and Stein, 2015).

Reducing the particle size of corn is a common practice because of its subsequent improvements on pig growth performance (Lancheros et al., 2020). Healy et al. (1994) demonstrated that reducing particle size from 900 to 300 μm during the first 14 days post weaning resulted in an improvement in Gain:Feed (G:F) and ADG. Improvements in G:F have also been demonstrated in finishing pigs as particle size was reduced from 1000 to 400 μm (Wondra et al., 1995b). These improvements in growth performance can be attributed to an increase in surface area to volume ratio of particles in the corn as particle size decreases. Therefore, this increase in surface area allows for greater enzyme activity which can lead to

improvements in utilization (Huang et al., 2015). Previous research has demonstrated that decreasing corn particle size (d_{gw}) from 1200 to 400 μm improves gross energy (GE), DE, ME, and nitrogen (N) digestibility in lactating sows (Wondra et al. 1995a). Healy and others (1994) saw a 2% increase in apparent total tract (ATTD) GE digestibility in nursery pigs. Similar improvements can also be seen in finishing pigs with ATTD GE improving by 3%, or 7% as particle size was reduced from 865 to 339 μm or 1000 to 400 μm respectively (Rojas and Stein, 2015, Wondra et.al. 1995b).

The majority of particle size research has been conducted using nursery and grow finish pigs, with little research on lactating sows, and little to no research on the effects of corn particle size on nutrient digestibility in gestating sows. Therefore, the objective of this paper was to determine the effects of corn particle size on the digestibility of dry matter (DM), protein (CP), and digestible energy (DE), metabolizable energy (ME), and nitrogen adjusted metabolizable energy (AMEn) in gestating sow diets.

Materials and 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 gestating sows (Line 241; DNA, Columbus, NE) of varying parity's were utilized in the study, during the second phase of their gestation (Day 40-74). 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-soy diet was formulated based

on the NRC 2012 suggestions, there were no ingredient inclusion changes across treatments (Table 4.1). 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 7 days to allow for adaptation to the treatment diets followed by a two-day collection period of fecal and urine samples.

Sample Collection

At the start of the collection period each sow was fitted with a Foley catheter (Bard Bardia 2-way, 30 mL balloon, 18 French; Bard Medical Canada Inc., Oakville, ON, Canada) with methods adapted from Holen et al. (2020). While sows were standing, the vulva region was cleaned with antiseptic solution (Betadine), and isopropyl alcohol. Technicians washed their hands before working with each sow with antiseptic soap and then wore sterile surgical gloves. Lubricant was placed onto the catheter and the hand of the technician before insertions. The lubricant was laced with lidocaine to prevent urethral spasms during placement. A total of 5 min was allotted for each placement to help prevent infection. Should a sow not be fitted with a catheter within the allotted time, she was removed from test. There were 9 sows placed on the 800 and 1200 μm treatments and 8 sows placed on the 400 μm trial as one sow was not able to be catheterized within the allotted placement time. The tip of the catheter was guided by the technician's finger along the floor of the vagina until it entered the urethra. Once the catheter was fully inserted the 30 mL balloon was inflated with 30 mL of saline solution to retain the catheter in the bladder. Polyvinyl tubing was connected to the catheters that dispensed the urine into a collection vessel. 20 mL of sulfuric acid was added to each bucket to keep the pH below 3

to limit bacterial growth and maintain nitrogen levels. Collection vessels were emptied, and subsamples were collected to obtain 20% aliquots. Aliquots were stored separately at -20 °C and subsamples were pooled within sow at the end of the trial. At the end of the 48-hour collection period the 30 mL balloon was deflated, and the catheter was removed. During collection periods sow behavior and vaginal discharge was evaluated twice daily. Sow temperatures were also collected twice daily during the collection period, and for 5 days following to monitor for any signs of urinary tract infection.

Fecal grab samples were collected throughout the collection period for each sow, bagged and stored separately at -20 °C. At the end of the trial, fecal samples were pooled within sow. All samples were stored -2 0°C until the end of the collection period. At the end of the trial samples were pooled within sow and subsamples were collected for 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).

To determine dry matter (DM) of feed, aluminum pans were weighed, and a ground sample was placed in the drying oven at 105 ˚C for 24 h. Samples were weighed back for moisture calculation (100 – ((dried sample weight / initial sample weight) × 100))) (AOAC Method 934.01). Fecal DM was determined in a two-step process. Samples were weighed and dried in a forced-air oven at 55 °C for 48 h. Samples were then weighed and moisture loss was recorded. Samples were ground and placed in Ziploc® plastic bags to be used for nutrient analysis and final DM analysis. To determine final dry matter of the fecal samples, aluminum pans were weighed, and a ground sample was weighed and placed in a forced air oven at 105°C

for 24 h in duplicate. Dried sample weights were recorded, and moisture levels were added from both drying steps to determine overall DM.

Fecal samples, urine, diets, and feed was analyzed for N via combustion using a Leco (Model FP928, St. Joseph, MI, Method 990.03; AOAC, 2007) nitrogen analyzer. Feed and fecal gross energy was determined via bomb calorimetry by ATC Scientific (North Little Rock, AR) using a Parr model 6100 (Parr Instrument Company, Moline, IL) bomb calorimeter. Urine samples were analyzed utilizing a Parr model 6200 (Parr Instrument Company, Moline, IL) bomb calorimeter using methods adapted from Jones (2015). To analyze urine for energy, cellulose was ground and dried at 105 ℃ for 24 h and stored in a sealed Ziplock bag until use. Then, 1 g of cellulose was placed into a bomb cup and 4 mL of urine was pipetted over the cellulose. The cup with cellulose and urine was dried for 12 h in a forced air oven at 55 ℃. Cups were removed from the oven and placed in a desiccator until they were bombed. Fecal and feed samples were analyzed for titanium dioxide via the methods described by J.L Leone (1973).

Calculations and Statistical Analysis

The following equation was used to calculate the digestibility energy of the diet using titanium dioxide as an indigestible marker. Gross energy of urine was then used to calculate ME from DE.

DE (kcal/kg)=(100 − (100 × (% TiO_2 in feed/% TiO_2 in feces) × (% nutrient in feces & urine/% nutrient in feed)))

(Agudelo, Lindemann, and Cromwell, 2010)

The ME was corrected for retained nitrogen (AMEn) by using the adjustment factor of 7.45 (Harris et.al., 1972) and the following calculation:

$$AMEn\ (kcal/kg) = ME-(7.46\times NR)$$

Where NR is the nitrogen retention, g/kg DMI (Zhang and Adeola, 2017).

The ME of corn was calculated by subtracting off the ME of soybean mean and soy oil utilizing the NRC 2012 values for these ingredients.

Data were analyzed using the PROC Glimmix procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) utilizing linear and quadratic polynomial contrast 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 $P \leq 0.10$.

Results and Discussion

Particle size reduction continues to be used in the swine industry to increase nutrient utilization regardless of production phase (Lancheros et al., 2020). Both roller mills and hammermills are utilized to grind grains. The type of mill used is commonly chosen by capacity, target particle size, and energy efficiency (Hancock and Behnke, 2001). Roller mills are more efficient than hammermills but cannot grind to as fine of a particle size as a hammermill. For the current trial, a 3 high roller mill was utilized to achieve the target particle sizes of 400, 800, and 1200 μm (Table 4.2). The geometric diameter average (dgw), or particle sizes of 403, 823, and 1372 μm, were achieved to correspond to the targeted treatments of 400, 800, and 1200 μm respectively. The geometric standard deviations (Sgw) for each treatment was 2.59, 2.80, and 2.83, respectively. When looking at the particle sizes of the diet, the dgw's were 448, 733, and 1155. The Sgw's of 2.52, 2.80, and 2.98 with respect to the 3 target particle sizes. As corn is ground to a coarser particle size, it is expected that the standard deviation will increase likely due to larger roll gaps in the larger particle size treatments that allow a wider range of particle sizes to flow through (Rojas and Stein, 2015, Wondra et al., 1995c). The target particle size of the diet

can vary from that of corn as the particle size of other ingredients often does not match that of corn (Wondra et al., 1995c).

Throughout the experiment, sows remained healthy with no signs of urinary tract infection related to being catheterized. One sow was removed from the experiment due to the catheter placement time exceeding the 5 min limit. All DE, ME, and AMEn calculations were conducted on a DM basis. When determining the ME and AMEn provided, these values were adjusted to an as is basis using a DM of 88.5%.

The ATTD of CP increased from 79% to 84% (linear, $P < 0.001$) as corn particle size decreased from 1200 to 400 μm (Table 4.3). Wondra et al. (1995a, and 1995c) observed similar improvements from 6-8% points in CP digestibility in sows when reducing corn particle size from 1200 to 400μm. Similarly, in both nursery and grow/finish phases, improvements in CP digestion have also been seen ranging from 3-17% depending on reduction in particle size (Acosta et.al., 2020, Healey et. al., 1994, Kim et.al., 2002). The improvement in CP digestion could be a result of particle size providing a greater surface area for protease activity (Ortschak et al., 2002). In contrast to what was seen in total tract digestibility, Rojas and Stein (2015) did not observe an improvement in ileal CP digestibility when reducing corn particle size in growing pigs. However, Owsley et al. (1981) determined that both the ileal and total tract CP digestibility of sorghum improved as sorghum particle size was reduced.

The ATTD of GE, DE, ME, AMEn, and ME of corn improved (linear, $P < 0.01$) as d_{gw} was decreased from 1200 to 400 μm. The experiment reported herein demonstrated a 170 and 178 kcal/kg increase in DE and ME, respectively. Wondra et al. (1995a) demonstrated double the increases in DE and ME with a 344 kcal/kg and a 346 kcal/kg increase respectively as corn particle size was reduced from 1200 to 400 μm when fed to lactating sows. When correcting ME

for nitrogen (AMEN, 88.5% DM) the dietary energy increased by 179 kcal/kg, which results in a 22.4 kcal/ kg improvement for every 100 μm in d_{gw} decrease in particle size. The ME value of corn was calculated by assuming the energy value of soy oil and soybean meal were 8,574 and 3,294 kcal/kg, respectively (NRC, 2012). Therefore, as the particle size of corn was reduced, the ME of corn (88.5% DM) increased (linear, *P = 0.009*) from 3,146 to 3,374 kcal/kg for the trial herein. The NRC (2012) reports that the ME value of corn is 3,395 kcal/kg, which is similar to the estimated ME of corn when ground to d_{gw} of 400 μm in the present study. In the study reported herein, it is estimated that for every 100 μm reduction in corn dgw the ME value of corn is increased by 28.5 kcal/kg. Bertol et.al. (2017) determined in growing-finishing pigs that as corn particle size was reduced from 982 to 525 μm, the AMEn value of corn increased by 50 kcal/kg for every 100 μm in d_{gw}. This value is greater than that observed in the trial herein. However, Rojas and Stein (2015) determined that the ME value of corn increased by 22.9 kcal/kg for every 100 μm reduction in corn dgw when fed to growing pigs. Improvements in energy digestibility are likely due to the starch portion of the corn having a larger surface area allowing for increased access for enzymes such as α-amylase (Huang et.al., 2015, Rojas and Stein, 2015).

Conclusion

In conclusion, as corn particle size decreases by 100 μm a 22.25 kcal.kg improvement in dietary ME (88.5% DM) was demonstrated in gestating sows. AMEn (88.5% DM) of the diet increased by 179 kcal/kg, which results in a 22.4 kcal/ kg improvement for every 100 μm decrease in particle size. Corn ME also increased in this trial with a 100 μm decrease in corn particle size resulting in a 28.5 kcal/kg improvement. Overall, the reduction of corn particle size

from 1200 to 400 μm resulted in a 5% improvement in CP digestibility and a 6% improvement in the dietary ME digestibility.

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Tables

Table 4-1: Diet composition, as-fed basis.

Ingredient, %	
Corn	78.15
Soybean meal, 46.5%	17.27
Soy oil	0.50
Monocalcium phosphate 21%	1.30
Limestone	1.30
Salt	0.50
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Sow add pack[3]	0.25
Phytase[4]	0.08
Titanium Dioxide	0.25
Total	100
Calculated Analysis[5], as-fed basis:	
ME, kcal/kg	3265
NE, kcal/kg	2486
CP, %	14.7
Ca, %	0.91
P, %	0.61
Ca:P	1.50
Analyzed Nutrients, as-fed basis	
DM, %	89.40
GE, kcal/kg	3887
CP, %	14.74

[1] 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.

[2] 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.

[3]Provided per kg of premix: 0.077 g chromium, 1,653,750 IU vitamin A from vitamin A acetate; 8820 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.

[4] 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

[5] Calculated Nutrient Values are based on NRC 2012 Values

Table 4-2:Particle size of corn and the final diet particle size. [1,2]

Target Corn Particle Size	400µm	800µm	1200µm
Corn Particle Size			
D_{gw}	403	823	1372
S_{gw}	2.59	2.80	2.83
Diet Particle Size			
D_{gw}	447	732	1154
S_{gw}	2.52	2.80	2.98

[1] Diets were ground on a 3 high RMS roller mill (Model 924, RMS Roller Grinder, Harrisburg, SD)

[2] Particle sizes were determined via the ANSI/ASAE method S319.2 (1995)) utilizing 0.5 g of sieving agents and a 10 min run time. .

Table 4-3: Effect of Corn Particle Size on Energy and Protein Digestibility of Gestating Sows. [1]

Corn Particle Size	400µm	800µm	1200µm	SEM	Linear	Quadratic
CP[2], %	84.1	82.3	79.1	0.997	0.001	0.553
GE[2], %	86.6	84.6	82.6	1.013	0.006	0.971
DE[3], kcal/kg	3,332	3,249	3,184	11.1	0.008	0.836
ME[3], kcal/kg	3,248	3,181	3,070	40.5	0.004	0.646
AMEn[3], kcal/kg	3196	3,125	3,017	40.3	0.004	0.699
Corn ME3, kcal/kg	3,374	3,288	3,146	51.9	0.004	0.646

[1] 27 gestating sows were used in a 9-day digestibility study, with a 7-day adjustment period and 2-day collection period. Sows were fed a common diet with three particle sizes of corn. Feed, Fecal, and Urine samples were collected and analyzed for DM, N, GE, and TiO_2 to determine digestibility.

[2]DE, ME, and Corn ME, values corrected for 88.5% DM

[3] Corn ME was Calculated by subtracting the ME of soybean meal and soy oil utilizing the NRC 2012 adjustment values.