



# SWINE DAY 2016

**K-STATE**  
Research and Extension

Kansas State University Agricultural Experiment Station and Cooperative Extension Service

# SWINE DAY 2016

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## Assessing the Effects of Medium Chain Fatty Acids and Fat Sources on Porcine Epidemic Diarrhea Virus Viral RNA Stability and Infectivity<sup>1</sup>

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### Summary

Research has confirmed that chemical treatments, such as medium chain fatty acids (MCFA) and commercial formaldehyde, can be effective to reduce the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination in feed. However, the efficacy of individual MCFA levels are unknown. The objective of this study is to compare the efficacy of commercially-available sources of MCFA and other fat sources versus a synthetic custom blend of MCFA to minimize the risk of PEDV cross-contamination as measured by qRT-PCR and bioassay. Treatments were arranged in a 17 × 4 plus 1 factorial with 17 chemical treatments: 1) Positive control with PEDV and no chemical treatment, 2) 0.3% Sal CURB, 3) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized), 4) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized), 5) 0.66% caproic acid, 6) 0.66% caprylic acid, 7) 0.66% capric acid, 8) 0.66% lauric acid, 9) 1% capric and lauric acid mixture (1:1 ratio), 10) FRA C12, 11) 1% choice white grease, 12) 1% soy oil, 13) 1% canola oil, 14) 2% palm kernel oil, 15) 1% palm kernel oil, 16) 2% coconut oil, and 17) 1% coconut oil; 4 analysis days of 0, 1, 3, and 7 post inoculation; and 1 treatment of PEDV negative, untreated feed. Matrices were first chemically treated, then inoculated with PEDV, and stored at room temperature until being analyzed by qRT-PCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ( $P < 0.002$ ). The interaction of treatment × day indicated that over time the MCFA treatments, either as a mixture or as individual fatty acids, and Sal CURB had the greatest effect of reducing detectable PEDV RNA, which follows the same trend as the main effect

<sup>1</sup> Appreciation is expressed to the National Pork Board for financial support (award #15-207).

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of treatment and the bioassay results. Feed treated with individual synthetic MCFA, MCFA mixture, or Sal CURB had fewer ( $P < 0.05$ ) detectable viral particles than all other treatments. Day also had a significant impact on quantification of viral RNA, and CT increased from 29.5 to 34.6 CT from day 0 to 7, respectively. In summary, time, Sal CURB, 1% MCFA, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids enhance the RNA degradation of PEDV in swine feed. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product in the complete swine diet at 1% inclusion and as individual fatty acids.

Key words: PEDV, medium chain fatty acids, fat source, swine

## Introduction

Porcine Epidemic Diarrhea Virus (PEDV) is an enveloped single-stranded positive-sense RNA virus that was first identified in the United States in May 2013. Epidemiological and controlled experiments have shown that complete feed or feed components can be one of many possible vectors of transmission of PEDV.<sup>5</sup> Previous research has shown that a 2% and 1% mixture of caproic, caprylic, and capric acids can reduce the risk of PEDV in a complete swine diet.<sup>6</sup> However, it has not been established if the response observed from the medium chain fatty acid (MCFA) treatment is due to unique characteristics of those particular fatty acids, or if the response is due to increasing the total quantity of fat in the diet. Furthermore, the synthetic blend of MCFA previously tested is not commercially available and may be cost-prohibitive to employ, so further evaluation of the mode-of-action of MCFA and potential replacement with commercially-available sources is warranted. Therefore, the objective of this study is to compare the efficacy of commercially available sources of MCFA and other fat sources versus a synthetic custom blend of MCFA to minimize the risk of PEDV cross-contamination as measured by qRT-PCR and bioassay.

## Procedures

In order to evaluate the use of chemical treatments and fat sources on PEDV survival, a corn-soybean meal-based swine diet was used and manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The diet was first chemically treated before inoculation with PEDV in order to mimic post-processing contamination.

## Chemical Treatment

Eighteen chemical treatments were applied to the diet and analyzed on 4 days (d 0, 1, 3, and 7 post inoculation). The 18 treatments were 1) negative control with no PEDV and no chemical; 2) positive control with PEDV and no chemical treatment; 3) 0.325% Sal CURB; Kemin Industries, Des Moines, IA; 4) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized); 5) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized); 6) 0.66% caproic acid; 7) 0.66% caprylic acid; 8) 0.66% capric acid; 9) 0.66% lauric acid; 10) 1% capric

<sup>5</sup> Dee et al., 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Veterinary Research 2014, 10:176.

<sup>6</sup> Cochrane et al., 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma." Journal of Animal Science 94.supplement2 (2016): 50-50.

and lauric acid mixture (1:1 ratio); 11) FRA C12; Framelco, Raamsdonksveer, Netherlands; 12) 1% choice white grease; 13) 1% soy oil; 14) 1% canola oil; 15) 2% palm kernel oil; 16) 1% palm kernel oil; 17) 2% coconut oil; and 18) 1% coconut oil.

In order to treat the feed, all treatments were added on a wt/wt basis and mixed using a lab scale paddle mixer. The Sal CURB and MCFA aerosolized treatments were mixed using an air atomizing nozzle in order to reduce the droplet size of the liquid treatments. The rest of the treatments were added directly to the mixer. All treatments were mixed for a 5-minute wet mix time to ensure a uniform and complete mix.

When the mixing was complete, a total of 22.5 g of product was collected from different locations within the mixer and added to the respective 250 mL HDPE, square, wide-mouth bottle based on day and replication. In order to reduce the potential for treatment-to-treatment cross-contamination, the mixers were cleaned with soap and water between treatments. Once the treatments were added to their respective bottle, they were allowed to sit at room temperature until inoculation.

### *PEDV Isolate*

The U.S. PEDV prototype strain cell culture isolate USA/IN/2013/19338, passage 8 (PEDV19338), was used to inoculate feed. Virus isolation, propagation, and titration were performed in Vero cells (ATCC CCL-81) as described by Chen et al. (2014).<sup>7</sup> The stock virus titer contained  $4.5 \times 10^6$  TCID<sub>50</sub>/mL and was diluted to  $10^5$  TCID<sub>50</sub>/mL.

### *Inoculation*

The feed was inoculated using an appropriately sized pipet to allow even distribution of the virus within the feed. For the inoculation, 2.5 mL of diluted viral inoculum was placed in each 250 mL bottle containing 22.5 grams of each feed treatment, resulting in each bottle containing a PEDV concentration of  $10^4$  TCID<sub>50</sub>/g of feed. The bottles were then thoroughly shaken to ensure equal dispersion of the virus within each bottle. The samples were then stored at ambient temperature until aliquoted for viral RNA expression of PEDV at 0, 1, 3, and 7 days post inoculation via qRT-PCR. For each sample day, 100 mL of chilled PBS was placed in each 250 mL bottle containing 22.5 g of inoculated feed. Samples were then shaken to thoroughly mix and chilled at 4°C overnight. Feed matrix supernatants, including two PCR samples and a bioassay sample, were then pulled and stored at -80°C until the end of the trial.

### *Bioassay*

The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol. Based on the qRT-PCR results, 15 treatments were selected for the bioassay. The 15 treatments were 1) d 0 negative control with no PEDV and no chemical treatment; 2) d 0 positive control with PEDV and no chemical treatment; 3) d 1 positive control with PEDV and no chemical treatment; 4) d 1 0.3% Sal CURB; 5) d 1 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized); 6) d 1 0.66% caproic acid; 7) d 1 0.66% caprylic acid; 8) d 1 0.66% capric acid; 9) d 1 0.66% lauric acid; 10) d 1 FRA C12; 11) d 1 1% choice white

<sup>7</sup> Chen et al., 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. *J. Clin. Microbiol.* 52: 234-243.

grease; 12) d 1 1% soy oil; 13) d 1 1% canola oil; 14) d 1 1% palm kernel oil; and 15) d 1 1% coconut oil.

A total of 45 crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Additionally, all pigs were confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) based on fecal swab. To further confirm PEDV-negative status, collected blood serum was analyzed for PEDV antibodies by an indirect fluorescent antibody (IFA) assay and TGEV antibodies by ELISA, both conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began. A total of 15 rooms (45 pigs) were assigned to treatment groups with 1 negative control room and 14 challenge rooms.

During bioassays, rectal swabs were collected on d -2, 0, 2, 4, 6, and 7 post inoculation (dpi) from all pigs and tested for PEDV RNA qRT-PCR. Following humane euthanasia at 7 dpi, small intestine, cecum, and colon samples were collected at necropsy along with an aliquot of cecal contents. One section of formalin-fixed proximal, middle, distal jejunum and ileum was collected per pig for histopathology.<sup>7</sup>

### *Statistical Analysis*

Data of the main effect of treatment, day, and the interaction were analyzed as a completely randomized design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC). Results for treatment criteria were considered significant at  $P \leq 0.05$  and marginally significant from  $P > 0.05$  to  $P \leq 0.10$ .

## **Results and Discussion**

### *qRT-PCR Results*

All main effects and interactions were significant at  $P < 0.0002$ . The results of the interaction between treatment and day show that the PEDV positive treatment, choice white grease (CWG), soy oil, canola oil, palm kernel oil (PKO), and coconut oil (CO) were all relatively stable over the 7 days (Table 1). However, when looking at the MCFA and Sal CURB treatments, it was observed that over time these treatments resulted in a greater reduction of the detectable genetic material ( $P < 0.05$ ). The MCFA non-aerosolized (40.0 CT) and aerosolized (39.0 CT) treatments did result in a greater reduction of detectable genetic material compared to Sal CURB (37.3 CT) over the 7 days ( $P < 0.05$ ).

The effect of day resulted in an increase of CT values from 29.5 to 34.6 over the 4-day experiment, with each of the analysis days being significant from one another ( $P < 0.0001$ ; Table 2). The increase in CT value also indicates that there is less detectable genetic material present.

The effect of treatment on PEDV was also significant and the resulting CT values varied from treatment to treatment ( $P < 0.0001$ ; Table 3). The treatments that resulted in the highest CT values, or less detectable genetic material, were the MCFA blends (non-aerosolized and aerosolized), caprylic acid, caprylic acid, and Sal CURB resulting

in significant differences of 6.9, 6.3, 5.8, 5.0, and 3.0 CT, respectively compared to the PEDV positive control ( $P < 0.05$ ). The FRA C12, CWG, soy oil, canola oil, PKO, and CO did not seem to have the same effect as the treatments mentioned above as they were statistically similar to the untreated control ( $P > 0.05$ ).

**Bioassay Results**

As expected Sal CURB resulted in a negative bioassay along with 1% MCFA mixture, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids (Table 4). In both instances the PEDV positive treatments at day 0 and 1 both resulted in positive bioassays along with each of the other day 1 treatments. However, it is important to point out that the coconut oil treatment was not deemed positive until the last day of the bioassay.

In summary, time, Sal CURB, 1% MCFA, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids enhance the RNA degradation of PEDV in swine feed such that infectivity was prevented. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product in the complete swine diet at 1% inclusion and as individual fatty acids.

**Table 1. Effect of treatment × day post inoculation on PEDV detection using RT-PCR<sup>1,2</sup>**

Item	Day				SEM	P =
	0	1	3	7		
PEDV positive	28.3 <sup>wvxyz</sup>	29.7 <sup>rstuv</sup>	31.3 <sup>nopq</sup>	32.7 <sup>klmn</sup>	0.5239	0.0002
Sal CURB <sup>3</sup>	28.7 <sup>uvwxy</sup>	33.0 <sup>ijklm</sup>	35.0 <sup>fgh</sup>	37.3 <sup>cd</sup>		
1% MCFA (aero)	33.3 <sup>ijkl</sup>	36.3 <sup>def</sup>	38.3 <sup>bc</sup>	39.0 <sup>ab</sup>		
1% MCFA (non-aero)	34.3 <sup>ghij</sup>	38.3 <sup>bc</sup>	37.0 <sup>cde</sup>	40.0 <sup>a</sup>		
0.66 % Caproic	33.7 <sup>hijk</sup>	35.0 <sup>fgh</sup>	36.3 <sup>def</sup>	37.0 <sup>cde</sup>		
0.66% Caprylic	34.3 <sup>ghij</sup>	35.7 <sup>efg</sup>	38.0 <sup>bc</sup>	37.3 <sup>cd</sup>		
0.66% Capric	29.3 <sup>stuvw</sup>	30.7 <sup>opqrs</sup>	34.0 <sup>ghij</sup>	35.3 <sup>fg</sup>		
0.66 % Lauric	28.3 <sup>vwxzy</sup>	30.7 <sup>opqrs</sup>	32.7 <sup>klmn</sup>	34.7 <sup>ghi</sup>		
1% Capric:Lauric	29.0 <sup>tuvwx</sup>	31.7 <sup>mnpq</sup>	34.3 <sup>ghij</sup>	34.3 <sup>ghij</sup>		
0.3% FRA C12 <sup>4</sup>	28.0 <sup>wxyz</sup>	30.7 <sup>opqrs</sup>	31.7 <sup>mnpq</sup>	33.7 <sup>hijk</sup>		
1% Choice white grease	28.3 <sup>vwxzy</sup>	30.0 <sup>qrstu</sup>	30.7 <sup>opqrs</sup>	32.0 <sup>lmno</sup>		
1% Soy	27.7 <sup>xyz</sup>	30.0 <sup>qrstu</sup>	30.3 <sup>pqrst</sup>	32.0 <sup>lmno</sup>		
1% Canola	27.0 <sup>z</sup>	30.7 <sup>opqrs</sup>	31.0 <sup>opqr</sup>	31.7 <sup>mnpq</sup>		
1% Palm kernel oil	27.7 <sup>xyz</sup>	30.0 <sup>qrstu</sup>	31.0 <sup>opqr</sup>	33.0 <sup>klmn</sup>		
2% Palm kernel oil	27.3 <sup>yz</sup>	29.7 <sup>rstuv</sup>	30.3 <sup>pqrst</sup>	33.0 <sup>klmn</sup>		
1% Coconut oil	28.0 <sup>wxyz</sup>	30.3 <sup>pqrst</sup>	31.3 <sup>nopq</sup>	32.7 <sup>klmn</sup>		
2% Coconut oil	27.3 <sup>yz</sup>	29.3 <sup>stuvw</sup>	29.7 <sup>rstuv</sup>	32.7 <sup>klmn</sup>		

<sup>1</sup> A total of 204 samples were used for the analysis with each treatment represented by a mean of N=3.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>3</sup> Kemin Industries, Des Moines, IA.

<sup>4</sup> Framelco, Raamsdonksveer, Netherlands.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 2. Main effect of day post-inoculation on detection of PEDV by qRT-PCR<sup>1</sup>**

Item	Day				SEM	P =
	0	1	3	7		
CT value <sup>2</sup>	29.5 <sup>a</sup>	31.9 <sup>b</sup>	33.1 <sup>c</sup>	34.6 <sup>d</sup>	0.13	<0.0001

<sup>1</sup> A total of 204 samples were used for the analysis with each day represented by a mean of N=51.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 3. Main effect of treatment prior to PEDV-inoculation on PEDV detection using RT-PCR<sup>1,2</sup>**

Item	CT Value	SEM	P =
PEDV positive	30.5 <sup>gh</sup>	0.262	<0.0001
Sal CURB <sup>3</sup>	33.5 <sup>d</sup>		
1% MCFA (aero)	36.8 <sup>ab</sup>		
1% MCFA (non aero)	37.4 <sup>a</sup>		
0.66 % Caproic	35.5 <sup>b</sup>		
0.66% Caprylic	36.3 <sup>c</sup>		
0.66% Capric	32.4 <sup>c</sup>		
0.66 % Lauric	31.6 <sup>f</sup>		
1% Capric:Lauric	32.3 <sup>c</sup>		
0.3% FRA C12 <sup>4</sup>	31.0 <sup>fg</sup>		
1% Choice white grease	30.3 <sup>hi</sup>		
1% Soy	30.0 <sup>hi</sup>		
1% Canola	30.1 <sup>hi</sup>		
1% Palm kernel oil	30.3 <sup>ghi</sup>		
2% Palm kernel oil	30.1 <sup>hi</sup>		
1% Coconut oil	30.6 <sup>gh</sup>		
2% Coconut oil	29.8 <sup>i</sup>		

<sup>1</sup> A total of 204 samples were used for the analysis with each treatment represented by a mean of N=12.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>3</sup> Kemin Industries, Des Moines, IA.

<sup>4</sup> Framelco, Raamsdonksveer, Netherlands.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 4. Effects of medium chain fatty acids, Sal CURB, and fat source on PEDV infectivity measured by pig fecal swabs and cecum content by qRT-PCR analysis<sup>1</sup>**

Item	PEDV N-gene Real Time-PCR, cycle threshold (CT)						
	Feed CT <sup>3</sup>	Fecal swabs					Cecum contents <sup>5</sup>
		0 dpi <sup>2</sup>	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
d 0							
PEDV negative	> 40.0	---	---	---	---	---	> 45.0
PEDV positive	28.3	---	-- +	+++	+++	+++	22.2
d 1							
PEDV positive	29.7	---	- ++	+++	+++	+++	20.9
Sal CURB <sup>6</sup>	33.0	---	---	---	---	---	> 45.0
1% MCFA (non-aero)	38.3	---	---	---	---	---	> 45.0
0.66 % Caproic	35.0	---	---	---	---	---	> 45.0
0.66% Caprylic	35.7	---	---	---	---	---	> 45.0
0.66% Capric	30.7	---	---	---	---	---	> 45.0
0.66 % Lauric	30.7	---	---	+++	+++	++	28.4
0.3% FRA C12 <sup>7</sup>	30.7	---	---	+++	+++	+++	30.2
1% Choice white grease	30.0	---	---	+++	+++	+++	15.3
1% Soybean oil	30.0	---	---	+++	+++	+++	24.0
1% Canola oil	30.7	---	---	+++	+++	+++	20.3
1% Palm kernel oil	30.0	---	---	+++	+++	+++	22.1
1% Coconut oil	30.3	---	---	---	---	++	42.1

<sup>1</sup> An initial tissue culture containing 10<sup>6</sup> TCID<sub>50</sub>/mL PEDV was diluted to 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV. Each treatment was inoculated with the 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV resulting in 10<sup>4</sup> TCID<sub>50</sub>/g PEDV inoculated feed matrix. Three feed samples per day and treatment were collected and diluted in PBS. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Thus, the cecum contents are represented by a mean of 3 pigs per treatment. Pigs were inoculated at d 12 age.

<sup>2</sup> Day post inoculation.

<sup>3</sup> A cycle threshold (CT of > 40) was considered negative for presence of PEDV RNA. Feed CT analysis was carried out at Kansas State University.

<sup>4</sup> In each instance a (-) signals a negative pig in the bioassay and a (+) represents a positive fecal swab in the bioassay. Each day post inoculation within each treatment has three symbols within each row and column which represents one of the three pigs in each treatment.

<sup>5</sup> A cycle threshold (CT of > 45) was considered negative for presence of PEDV RNA. Cecum content analysis was carried out at Iowa State University.

<sup>6</sup> Kemin Industries, Des Moines, IA.

<sup>7</sup> Framelco, Raamsdonksveer, Netherlands.

## Evaluating the Inclusion Level of Medium Chain Fatty Acids to Reduce the Risk of Porcine Epidemic Diarrhea Virus in Complete Feed and Spray-Dried Animal Plasma<sup>1</sup>

*R.A. Cochran, S.S. Dritz,<sup>2</sup> J.C. Woodworth, A.R. Huss,<sup>3</sup> C.R. Stark,<sup>3</sup> M. Saensukjaroenphon,<sup>3</sup> J.M. DeRouchey, M.D. Tokach, R.D. Goodband, J. Bia,<sup>2</sup> Q. Chen,<sup>4</sup> J. Zhang,<sup>4</sup> P.C. Gauger,<sup>4</sup> R.J. Derscheid,<sup>4</sup> D.R. Magstadt,<sup>4</sup> P. Arruda,<sup>4</sup> A. Ramirez,<sup>4</sup> R.G. Main,<sup>4</sup> and C.K. Jones*

### Summary

Research has confirmed that chemical treatments, such as medium chain fatty acids (MCFA) and commercial formaldehyde, can be effective to reduce the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination in feed. However, the efficacy of MCFA levels below 2% inclusion is unknown. The objective of this experiment was to evaluate if a 1% inclusion of MCFA is as effective at PEDV mitigation as a 2% inclusion or formaldehyde in swine feed and spray-dried animal plasma (SDAP). Treatments were arranged in a 4 × 2 × 7 plus 2 factorial with 4 chemical treatments: 1) PEDV positive with no chemical treatment, 2) 0.325% commercial formaldehyde, 3) 1% MCFA, and 4) 2% MCFA. The 2 matrices were: 1) complete swine diet and 2) SDAP; with 7 analysis days: 0, 1, 3, 7, 14, 21, and 42 post inoculation; and 1 treatment each of PEDV negative untreated feed and plasma. Matrices were first chemically treated, then inoculated with PEDV, and stored at room temperature until being analyzed by RT-qPCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ( $P < 0.009$ ). Feed treated with MCFA, regardless of inclusion level, had fewer ( $P < 0.05$ ) detectable viral particles than feed treated with formaldehyde. However, the SDAP-treated with either 1% or 2% MCFA had similar ( $P > 0.05$ ) concentrations of detectable PEDV RNA as the untreated SDAP, while the SDAP treated with formaldehyde had fewer detectable viral particles ( $P < 0.05$ ). The complete feed had a lower ( $P < 0.05$ ) quantity of PEDV RNA than SDAP (39.5 vs. 35.0 for feed vs. SDAP, respectively) ( $P$

<sup>1</sup> Appreciation is expressed to the National Pork Board for financial support (award #16-062).

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< 0.05). Analysis day also decreased ( $P < 0.05$ ) the quantity of detectable viral particles from d 0 to 42, (33.2 vs. 44.0, respectively). In summary, time, formaldehyde, and MCFA all appear to enhance RNA degradation of PEDV in swine feed and ingredients; however, their effectiveness varies within matrix. The 1% inclusion level of MCFA was as effective as 2% in complete feed, but neither were effective at reducing the magnitude of PEDV RNA in SDAP.

Key words: PEDV, medium chain fatty acids, feed matrix, swine

## Introduction

Porcine Epidemic Diarrhea Virus (PEDV) is an enveloped single-stranded positive-sense RNA virus that was first identified in the United States in May 2013. Epidemiological and controlled experiments have shown that complete feed or feed components can be one of many possible vectors of transmission of PEDV.<sup>5</sup> Because of the potential viral spread by feed and ingredients, reduction techniques such as chemical treatments have been used to combat the virus. Many chemical treatments have been used to mitigate the virus, but formaldehyde and Medium Chain Fatty Acids (MCFA) seem to have the greatest reduction of the virus within feed and ingredients. Formaldehyde has shown to be effective at the approved rate of addition (37% formaldehyde used in animal feed at rate of 5.4 lb per ton), and MCFA at 2% wt/wt in the feed or ingredient.<sup>6,7,8</sup> However, the efficacy of MCFA levels below 2% inclusion is unknown. Therefore, objective of this experiment was to evaluate if a 1% inclusion of MCFA is as effective at PEDV mitigation as a 2% inclusion or formaldehyde in swine feed and spray-dried animal plasma (SDAP).

## Procedures

In order to evaluate the use of chemical treatments on PEDV survival, a corn-soybean meal-based swine diet manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan Kansas, and spray-dried animal plasma were utilized. The feed matrices were first chemically treated before inoculation with PEDV in order to mimic post-processing contamination.

## Chemical Treatment

In order to evaluate the chemical treatments a  $4 \times 2 \times 7$  plus 2 factorial was utilized. The four chemical treatments; 1) positive control with PEDV and no chemical treatment, 2) 0.3% Sal CURB; Kemin Industries, Des Moines, IA, 3) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1; Sigma Aldrich, St. Louis, MO] (aerosolized), and 4) 2% medium chain fatty acid blend [caproic, caprylic, and

<sup>5</sup> Dee et al., 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Veterinary Research 2014, 10:176.

<sup>6</sup> Dee et al., 2015. An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial. Porcine Health Management. 1:9. doi, 10.1186/s40813-015-0003-0.

<sup>7</sup> Formaldehyde. 2003. 21 CFR § 573.460.

<sup>8</sup> Cochrane et al., 2015. Evaluating chemical mitigation of Porcine Epidemic Diarrhea virus in swine feed and ingredients. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

capric acids; 1:1:1] (aerosolized). These treatments were applied to 2 feed matrices 1) corn soybean meal-based swine diet and 2) spray-dried animal plasma, and evaluated on 7 analysis days (d 0, 1, 3, 7, 14, 21, and 42 post inoculation). There was also 1 treatment each of PEDV negative untreated feed and plasma, which acted as controls.

In order to treat the complete feed and plasma, all treatments were added on a wt/wt basis and mixed using a lab-scale paddle mixer. The Sal CURB and MCFA treatments were aerosolized into the mixer using an air-atomizing nozzle in order to reduce the droplet size of the liquid treatments. All treatments were mixed for a 5-minute wet mix time to ensure a uniform and complete mix.

Once the mixing was complete, a total of 22.5 g of product was collected from different locations within the mixer and added to the respective 250 mL HDPE, square, wide-mouth bottle based on day and replication. In order to reduce the potential for treatment-to-treatment cross-contamination, the mixer was cleaned with soap and water between treatments. Once the treatments were added to their respective bottle, they were allowed to sit at room temperature until inoculation.

### *PEDV Isolate*

The U.S. PEDV prototype strain cell culture isolate USA/IN/2013/19338, passage 8 (PEDV19338) was used to inoculate feed. Virus isolation, propagation, and titration were performed in Vero cells (ATCC CCL-81) as described by Chen et al. (2014).<sup>9</sup> The stock virus titer contained  $4.5 \times 10^6$  TCID<sub>50</sub>/mL and was diluted to  $10^5$  TCID<sub>50</sub>/mL.

### *Inoculation*

The feed was inoculated using an appropriately sized pipet to allow even distribution of the virus within the feed and plasma. For the inoculation, 2.5 mL of diluted viral inoculum was placed in each 250 mL bottle containing 22.5 grams of each feed treatment, resulting in each bottle containing a PEDV concentration of  $10^4$  TCID<sub>50</sub>/g of feed. The bottles were then thoroughly shaken to ensure equal dispersion of the virus within each bottle. The samples were then stored at ambient temperature until aliquoted for viral RNA expression of PEDV at 0, 1, 3, 7, 14, 21, and 42 days post treatment via qRT-PCR. For each sample day, 100 mL of chilled PBS was placed in each 250 mL bottle containing 22.5 g of inoculated feed. Samples were then shaken to thoroughly mix and chilled at 4°C overnight. Feed matrix supernatants, including two PCR samples and a bioassay sample, were then collected and stored at -80°C until the end of the trial.

### *Bioassay*

The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol. A total of 60 crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Additionally, all pigs were confirmed negative for PEDV, porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV) based on fecal swab. To further confirm PEDV negative status, collected blood serum was analyzed for PEDV antibodies by an indirect fluorescent antibody (IFA) assay and TGEV

<sup>9</sup> Chen et al., 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. *J. Clin. Microbiol.* 52: 234-243.

antibodies by ELISA, both conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began. A total of 20 rooms (60 pigs) were assigned to treatment groups with 2 negative control rooms and 18 challenge rooms. During bioassays, rectal swabs were collected on d -2, 0, 2, 4, 6, and 7 days post inoculation (dpi) from all pigs and tested for PEDV RNA qRT-PCR. Following humane euthanasia at 7 dpi, small intestine, cecum, and colon samples were collected at necropsy along with an aliquot of cecal contents. One section of formalin-fixed proximal, middle, distal jejunum and ileum was collected per pig for histopathology.<sup>9</sup>

### *Statistical Analysis*

Data of the main effects day, treatment, feed matrix, and all associated interactions were analyzed as a completely randomized design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC). Results for treatment criteria were considered significant at  $P \leq 0.05$  and marginally significant from  $P > 0.05$  to  $P \leq 0.10$ .

## **Results and Discussion**

### *qRT-PCR Results*

All main effects and interactions were highly significant ( $P < 0.0037$ ). Overall, Sal CURB and MCFA both differed from the control ( $P < 0.05$ ). Sal CURB was the most effective chemical treatment (CT = 38.3), followed by the 2% MCFA (CT = 38.2), and 1% MCFA (CT = 38.0), all of which reduced ( $P < 0.05$ ) the quantity of detectable PEDV nucleic acid compared to the PEDV positive untreated control (CT = 34.6) as detected by qRT-PCR (Table 1).

Significant differences were also observed between each of the feed matrixes ( $P < 0.0001$ ). Overall, complete diet had the greater PEDV CT, or less genetic material present (CT = 39.5), compared to the spray-dried animal plasma (CT = 35.0; Table 2).

Time also affected PEDV concentration detected by RT-PCR, with d 0 and 1 being statistically similar (33.2 vs. 34.3 CT, respectively;  $P > 0.05$ ), but lower ( $P < 0.05$ ) than d 3 (CT = 35.9 Table 3). The CT increased over time during d 3, 7, 14, 21, and 42 ( $P < 0.05$ ; 35.9, 36.5, 38.0, and 39.0, and 44.0 respectively).

Interactions are presented graphically and provide more relevant results regarding the effects of specific chemical mitigants in the complete diet and spray-dried animal plasma over time. The PEDV CT in the untreated control of the complete diet increased in a linear fashion from d 0-42 (Figure 1). The chemical treatments all had a greater decrease in detectable PEDV RNA at each analysis day than the untreated control. In the complete swine diet, the MCFA treatments regardless of concentration were the most effective overall.

The PEDV CT in the untreated control of the spray-dried animal plasma had the same trend for both MCFA treatments (Figure 2). However, the commercial formaldehyde product was highly successful at mitigating PEDV according to qRT-PCR in spray-dried animal plasma compared to the MCFA treatments.

**Bioassay Results**

The bioassay provided a more in-depth look at each of the chemical treatments as to which treatments led to no infection in the animals. In the complete feed, the only treatment that led to PEDV positive pigs was the day 0 PEDV positive feed with no chemical treatments (Table 4). However, the spray-dried animal plasma Sal CURB was the only treatment that led to a negative bioassay on d 3 (Table 5). On d 21 the Sal CURB, 1% MCFA, and PEDV positive untreated control all led to negative bioassays with the 2% MCFA treatment producing a positive bioassay 4 days post inoculation (Table 5).

In summary, time, Sal CURB, and MCFA enhance the RNA degradation of PEDV in swine feed and ingredients, but their effectiveness varies within matrix. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially available formaldehyde product in the complete swine diet at 1% inclusion.

**Table 1. Main effect of treatment on detection of PEDV by qRT-PCR<sup>1</sup>**

Item	PEDV pos.	Sal CURB	1% MCFA	2% MCFA	SEM	P =
CT value <sup>2</sup>	34.6 <sup>b</sup>	38.3 <sup>a</sup>	38.0 <sup>a</sup>	38.2 <sup>a</sup>	0.43	<0.0001

<sup>1</sup> A total of 168 samples were used for the analysis with each treatment represented by a mean of N=42.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 2. Main effect of feed matrix post inoculation on detection of PEDV by qRT-PCR<sup>1</sup>**

Item	Feed	SDAP	SEM	P =
CT value <sup>2</sup>	39.5 <sup>a</sup>	35.0 <sup>b</sup>	0.43	<0.0001

<sup>1</sup> A total of 168 samples were used for the analysis with each day represented by a mean of N=84.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 3. Main effect of day post inoculation on detection of PEDV by qRT-PCR<sup>1</sup>**

Item	Day							SEM	P =
	0	1	3	7	14	21	42		
CT value <sup>2</sup>	33.2 <sup>e</sup>	34.3 <sup>e</sup>	35.9 <sup>d</sup>	36.5 <sup>cd</sup>	38.0 <sup>bc</sup>	39.0 <sup>b</sup>	44.0 <sup>a</sup>	0.13	<0.0001

<sup>1</sup> A total of 168 samples were used for the analysis with each day represented by a mean of N=24.

<sup>2</sup> Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

<sup>ab</sup> Means within a row lacking a common superscript differ.

**Table 4. Effects of medium chain fatty acids and formaldehyde treatment of complete diet on porcine epidemic diarrhea virus (PEDV) detection from feed, pig fecal swabs and cecum contents<sup>1</sup>**

Item	PEDV N-gene Real Time-PCR, cycle threshold (Ct)						
	Feed CT	Fecal swabs					Cecum contents <sup>5</sup>
		0 dpi <sup>2</sup>	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
Unprocessed virus-free feed	> 45.0 <sup>3</sup>	---	---	---	---	---	> 45.0
Day 0 inoculated feed	31.0	---	---	- + -	++ -	++ -	28.0
Day 3 inoculated feed	34.1	---	---	---	---	---	> 45.0
Day 3 Sal CURB	37.2	---	---	---	---	---	> 45.0
Day 3 1% MCFA	42.8	---	---	---	---	---	> 45.0
Day 3 2% MCFA	42.4	---	---	---	---	---	> 45.0
Day 21 inoculated feed	37.3	---	---	---	---	---	> 45.0
Day 21 Sal CURB	40.4	---	---	---	---	---	> 45.0
Day 21 1% MCFA	> 45.0	---	---	---	---	---	> 45.0
Day 21 2% MCFA	> 45.0	---	---	---	---	---	> 45.0

<sup>1</sup> An initial tissue culture containing 10<sup>6</sup> TCID<sub>50</sub>/mL PEDV was diluted to 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV. Each treatment was inoculated with the 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV resulting in 10<sup>4</sup> TCID<sub>50</sub>/g PEDV inoculated feed matrix. Three feed samples per day and treatment were collected and diluted in PBS. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Pigs were inoculated at d 12 age.

<sup>2</sup> Day post inoculation.

<sup>3</sup> A cycle threshold (Ct) of >45 was considered negative for presence of PEDV RNA. Feed CT values were analyzed at Kansas State University.

<sup>4</sup> In each instance a (-) signals a negative pig in the bioassay and a (+) represents a positive in the bioassay. Each day post inoculation within each treatment has three symbols with each row and column, which represents one of the three pigs in each treatment.

<sup>5</sup> Each cecum content value represents the mean of 3 pigs per treatment and was analyzed at Iowa State University.

**Table 5. Effects of medium chain fatty acids and formaldehyde treatment of spray-dried porcine plasma on porcine epidemic diarrhea virus (PEDV) detection from plasma, pig fecal swabs and cecum contents<sup>1</sup>**

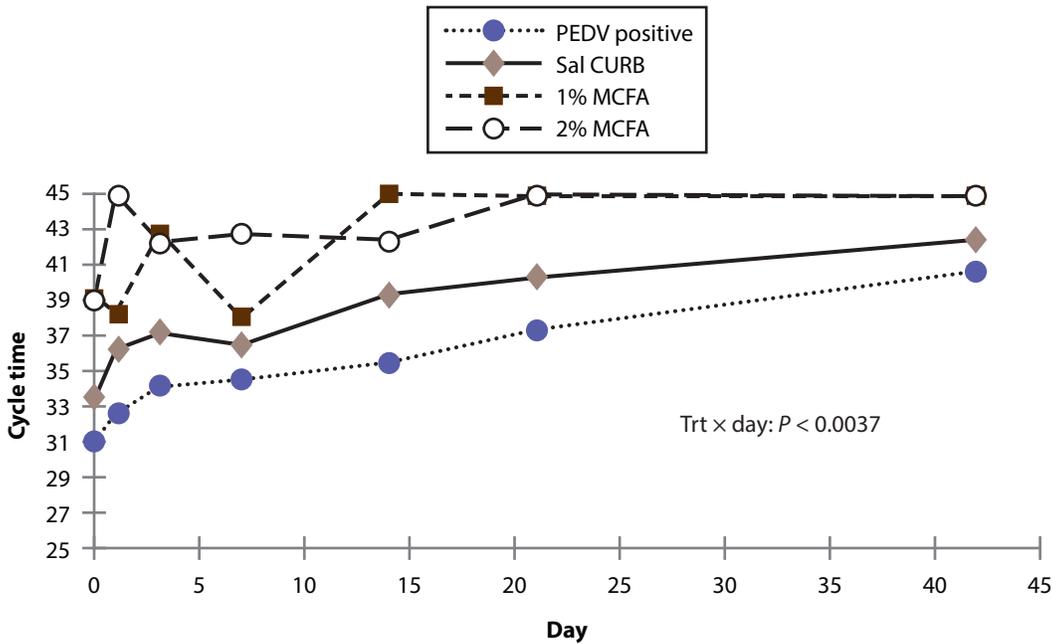
Item	PEDV N-gene Real Time-PCR, cycle threshold (Ct)						
	Plasma	Fecal swabs					Cecum contents
	CT	0 dpi <sup>2</sup>	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
Unprocessed virus-free feed	> 45.0 <sup>3</sup>	---	---	---	---	---	> 45.0
Day 0 inoculated plasma	30.1	---	+++	+++	+++	+++	27.8
Day 3 inoculated plasma	31.6	---	+++	+++	+++	+++	29.8
Day 3 Sal CURB	34.5	---	---	---	---	---	> 45.0
Day 3 1% MCFA	34.0	-	+++	+++	+++	+++	30.4
Day 3 2% MCFA	31.1	-	+++	+++	+++	+++	29.4
Day 21 inoculated plasma	36.0	---	---	---	---	---	> 45.0
Day 21 Sal CURB	> 45.0	---	---	---	---	---	> 45.0
Day 21 1% MCFA	31.7	---	---	---	---	---	> 45.0
Day 21 2% MCFA	31.5	---	---	- + -	+++	+++	31.3

<sup>1</sup> An initial tissue culture containing 10<sup>6</sup> TCID<sub>50</sub>/mL PEDV was diluted to 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV. Each treatment was inoculated with the 10<sup>5</sup> TCID<sub>50</sub>/mL PEDV resulting in 10<sup>4</sup> TCID<sub>50</sub>/g PEDV inoculated feed matrix. Three feed samples per day and treatment were collected and diluted in PBS. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Thus, each value represents the mean of 3 pigs per treatment. Pigs were inoculated at d 12 age.

<sup>2</sup> Day post inoculation.

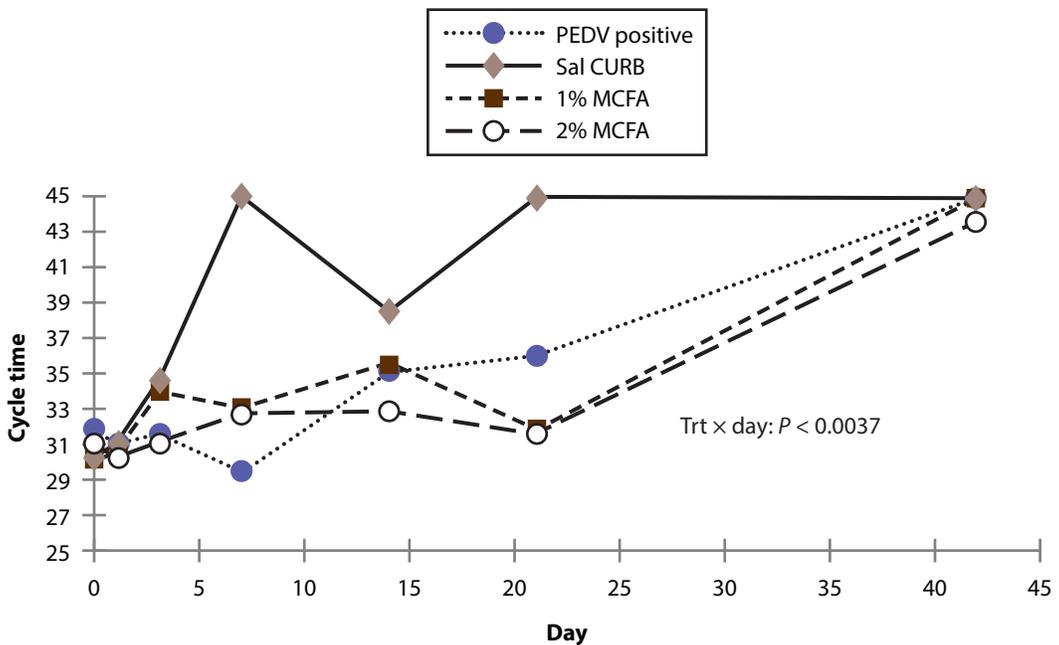
<sup>3</sup> A cycle threshold (Ct) of >45 was considered negative for presence of PEDV RNA.

<sup>4</sup> In each instance a (-) signals a negative pig in the bioassay and a (+) represents a positive in the bioassay. Each day post inoculation within each treatment has three symbols with each row and column, which represents one of the three pigs in each treatment.



**Figure 1. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated complete swine diet stored at room temperature.**

Data were analyzed by PCR with each data point represented by N=3. The higher the CT value, the less quantity of PEDV RNA genetic material is detected.



**Figure 2. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated spray-dried animal plasma stored at room temperature.**

Data were analyzed by PCR with each data point represented by N=3. The higher the CT value, the less quantity of PEDV RNA genetic material is detected.

## Porcine Epidemic Diarrhea Virus Surface Decontamination Strategies Using Chemical Sanitizing to Reduce the Quantity of PEDV RNA on Feed Manufacturing Surfaces with Environmental Swabbing<sup>1,2</sup>

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### Summary

Porcine Epidemic Diarrhea virus (PEDV) is a possible hazard in feed mills that could impact pig health. If the virus enters a feed mill, it quickly becomes widely distributed and is difficult to decontaminate from surfaces.<sup>6,7</sup> The objective of this study was to evaluate a variety of liquid and dry chemical treatments that could be used as sanitizers to reduce the amount of PEDV found on feed manufacturing surfaces in mills. This experiment was replicated 3 times and was designed in a 5 × 10 factorial with main effects of 5 different feed manufacturing surfaces and 10 sanitizing treatments. Surfaces included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete coupons (4 × 4 in). One mL (1 × 10<sup>5</sup> TCID<sub>50</sub>/mL) of stock PEDV was applied to each surface and allowed to dry completely for 60 min. Next, a mitigation treatment was applied for 15 min: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVital and CRINA; DSM Nutritional Products Inc., Parsippany, NJ); 6)

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<sup>6</sup> Schumacher, L.L., R.A. Cochrane, C.E. Evans, J.R. Kalivoda, J.C. Woodworth, C.R. Stark, C.K. Jones, Q. Chen, R.G. Main, J. Zhang, P.C. Gauger, S.S. Dritz, and M.D. Tokach. 2016. Evaluating the effect of manufacturing porcine epidemic diarrhea virus (PEDV)-contaminated feed on subsequent feed mill environmental surface contamination. *J. Anim. Sci.* 99(E2)164.

<sup>7</sup> Bowman, A. S., Nolting, J. M., Nelson, S. W., Bliss, N., Stull, J. W., Wang, Q., and Premanandan, C. 2015. Effects of disinfection on the molecular detection of porcine epidemic diarrhea virus. *Veterinary Microbiology*, 179(3), 213-218.

liquid ammonium chloride, isopropanol, and hydrogen peroxide-based commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN); 7) liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada); 8) liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV); 9) liquid sodium hypochlorite commercial sanitizer (Bleach; Clorox, Oakland, CA); and 10) liquid medium chain fatty acid blend of caprylic, caproic, and capric acids. There were 3 replicates per treatment. The quantity of PEDV RNA was determined using qRT-PCR. All main effects, interaction, and comparisons were highly significant ( $P \leq 0.001$ ). Liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive.

Key words: feed manufacturing, chemical sanitation, PEDV

## Introduction

The swine feed mill may be a potential vector for Porcine Epidemic Diarrhea virus (PEDV) transmission into swine herds.<sup>8,9,10</sup> Recent studies have demonstrated the potential for PEDV to be introduced to the feed mill through ingredients, vehicles, and employees.<sup>11</sup> Regardless of the method of entry, viral contamination becomes widespread once within the manufacturing environment due to dust contamination.<sup>6,12</sup> There are limited options to decontaminate feed mills once viral RNA has become established. Thermal processing inactivates the virus at 130°F.<sup>13</sup> However, it does not prevent re-contamination from PEDV-contaminated dust or residue on feed manufacturing equipment surfaces prior to loadout. Chemical sanitizers are typically used

<sup>8</sup> Schumacher, L.L., Cochrane, R.A., Evans, C.E., Kalivoda, J.R., Woodworth, J.C., Stark, C.R., Jones, C.K., Main, R.G., Zhang, J., Dritz, S.S. and Gauger, P.C., 2015. Evaluating the Effect of Manufacturing Porcine Epidemic Diarrhea Virus (PEDV)-Contaminated Feed on Subsequent Feed Mill Environmental Surface Contamination. Kansas Agricultural Experiment Station Research Reports, 1(7), p. 4.

<sup>9</sup> Greiner, Laura L. 2016. Evaluation of the likelihood of detection of porcine epidemic diarrhea virus or porcine delta coronavirus ribonucleic acid in areas within feed mills. Journal of Swine Health and Production. 24.4 198-204.

<sup>10</sup> Pasick, J., Berhane, Y., Ojkic, D., Maxie, G., Embury-Hyatt, C., Swekla, K., and Alexandersen, S. 2014. Investigation into the Role of Potentially Contaminated Feed as a Source of the First-Detected Outbreaks of Porcine Epidemic Diarrhea in Canada. Transboundary and emerging diseases, 61(5), 397-410.

<sup>11</sup> Cochrane, R.A., Dritz, S.S., Woodworth, J.C., Stark, C.R., Huss, A.R., Cano, J.P., Thompson, R.W., Fahrenholz, A.C. and Jones, C.K., 2016. Feed mill biosecurity plans: A systematic approach to prevent biological pathogens in swine feed. Journal of Swine Health and Production 24.3: 154-164.

<sup>12</sup> Gebhardt J. T., Woodworth J. C., Jones C. K., Gauger P. C., Tokach, M. D., DeRouche J. M., Goodband, R. D., Muckey M., Cochrane R. A., Stark C. R., Bai J., Chen Q., Zhang J., Ramirez A., Derscheid R. J., Main R. G., and Dritz S. S. 2016. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on likelihood of porcine epidemic diarrhea virus (PEDV) transmission by swine feed and feed manufacturing equipment. In Kansas State University Swine Day 2016. Kansas Agricultural Experiment Station Research Reports.

<sup>13</sup> Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouche, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, Jianqiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)-Contaminated Feed. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

for such purposes in human food manufacturing and have shown some promise on reducing PEDV RNA on trailer surfaces. Current industry practices include the use of heat, sodium hypochloride, or quaternary ammonium/glutaraldehyde combinations to sanitize swine farm surfaces contaminated with PEDV. However, there is limited information regarding their success on reducing viral RNA on feed manufacturing surfaces. Even if there were successful options, there may be limited application of liquid sanitizers due to the inherent dry nature of ingredients and feed. The introduction of water, even in the form of a liquid sanitizer, may actually increase the quantity of other biological hazards if they are not targeted by the sanitizer. Furthermore, ideal sanitizers would be safe for use in both the animal feed and on the equipment surface. The objective of this study was to evaluate the ability of a variety of liquid and dry chemical sanitizers to reduce the quantity of detectable PEDV RNA.

## Procedures

The experimental treatments were arranged as a  $5 \times 10$  factorial with 5 different feed manufacturing surfaces and 10 chemical treatments. Each combination was replicated 3 times. Surfaces included: 1) stainless steel (stainless steel type 316; Built-So-Well Manhattan, KS); 2) plastic (Dura Bucket National Oats Co. Collinsville, Ill.); 3) rubber (Maxi-Lift Inc. Addison, TX); 4) woven polypropylene tote bag (The MegaSack Corp. Magnolia, AR); and 5) sealed concrete (Quikrete Co. Atlanta, GA). Chemical treatments included: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVital and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend); 6) liquid commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride); 7) 3% dilution of liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide); 8) 0.39% dilution of liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde); 9) 10% dilution of liquid sodium hypochlorite commercial sanitizer (Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite); and 10) liquid medium chain fatty acid blend of caprylic, caproic, and capric acids (1:1:1 custom blend<sup>11</sup>).

A  $4 \times 4$  in. coupon of each surface was prepared, inoculated, and treated with chemical as previously described.<sup>7</sup> Briefly, surfaces were sanitized, rinsed, and autoclaved. Next, 1 mL of PEDV (USA/IN/2013/19338;  $1 \times 10^5$  TCID<sub>50</sub>/ml) was applied to the surfaces and spread using cell spreader to cover the entire area. Surfaces were allowed to dry for 60 min. After drying of PEDV, respective treatment was applied to coupon surface for 15 min.

Surfaces were then swabbed to determine residual PEDV contamination using pre-moistened environmental swabs in 5 mL of neutralizing broth (World Bioproducts LLC., Mundelein, IL). Swabs were vortexed and PEDV was quantified using qRT-

PCR. Results were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC). A preplanned contrast included the comparison of dry vs. liquid chemical treatments. Significance was considered at  $P \leq 0.05$  and marginally significant from  $P > 0.05$  to  $P \leq 0.10$ .

## Results and Discussion

All main effects and interactions were highly significant ( $P \leq 0.001$ ; Table 1 and 2). Rubber belting obtained from a bucket elevator retained the most PEDV RNA of any tested surface, while the polyethylene tote bag retained the least ( $P < 0.05$ ; 28.0 vs. 31.4 CT for rubber vs. tote bag, respectively). Concentrated liquid Sal CURB was the most effective sanitizer at removing PEDV RNA across surfaces, followed by liquid bleach ( $P < 0.05$ ; 42.9 vs. 35.2 CT for Sal CURB vs. bleach, respectively). The liquid Sal CURB prevented detection of PEDV RNA ( $> 45$  CT) on plastic, polyethylene tote bag, rubber, and stainless steel. Cement still contained residual PEDV RNA, even after liquid formaldehyde application, but the sanitizer was still more effective than other treatments ( $P < 0.05$ ; 36.7 CT). Liquid bleach was most effective at reducing PEDV RNA on the polyethylene tote bag (43.0 CT), followed by stainless steel, rubber, and plastic (37.1, 35.6, and 35.0 CT, respectively). Liquid bleach was least effective on cement ( $P < 0.05$ ; 25.4 CT). All other sanitizers did not influence the detection of PEDV RNA on any surface compared to that detected on the untreated control ( $P > 0.05$ ). Due to the performance of liquid Sal CURB and liquid bleach, liquid sanitizers were substantially more effective at reducing the quantity of detectable PEDV RNA compared to dry sanitizers ( $P < 0.05$ ).

In summary, liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive.

**Table 1. Main effects of different chemical treatments to reduce the quantity of PEDV RNA on feed manufacturing equipment surfaces with environmental swabbing<sup>1</sup>**

	PEDV, CT
Surface	
Cement	30.0 <sup>ab</sup>
Plastic	28.5 <sup>bc</sup>
Polyethylene tote bag	31.4 <sup>a</sup>
Rubber	28.0 <sup>c</sup>
Stainless steel	28.9 <sup>bc</sup>
Chemical treatment	
Untreated control	26.2 <sup>c</sup>
Untreated rice hulls	26.7 <sup>c</sup>
Commercial formaldehyde-treated rice hulls (2 kg/ton) <sup>2</sup>	26.2 <sup>c</sup>
Concentrated commercial formaldehyde <sup>2</sup>	42.9 <sup>a</sup>
Concentrated dry commercial benzoic acid and probiotic blend <sup>3</sup>	27.9 <sup>c</sup>
Ready-to-use liquid commercial food-grade sanitizer <sup>4</sup>	26.2 <sup>c</sup>
3% dilution of liquid hydrogen peroxide commercial product <sup>5</sup>	26.5 <sup>c</sup>
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product <sup>6</sup>	28.4 <sup>c</sup>
10% dilution of liquid sodium hypochlorite commercial sanitizer <sup>7</sup>	35.2 <sup>b</sup>
Concentrated liquid medium chain fatty acid blend <sup>8</sup>	27.4 <sup>c</sup>
<i>P</i> =	
Surface	0.001
Treatment	< 0.0001
Surface × treatment	0.001
Dry vs. liquid treatment	< 0.0001
SEM	
Surface	0.60
Treatment	0.85
Surface × treatment	1.91

<sup>1</sup> This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.

<sup>2</sup> Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.

<sup>3</sup> VevoVital and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.

<sup>4</sup> DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride.

<sup>5</sup> INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide.

<sup>6</sup> Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde.

<sup>7</sup> Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite.

<sup>8</sup> Caprylic, caproic, and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.

<sup>abc</sup> Means with different superscripts differ (*P* < 0.05).

**Table 2. Interaction of chemical treatments and feed manufacturing equipment surfaces to reduce the quantity of PEDV RNA with environmental swabbing<sup>1</sup>**

	Surface type				
	Cement	Plastic	Polyethylene tote bag	Rubber	Stainless steel
Chemical treatment					
Untreated control	27.5 <sup>fghij</sup>	26.7 <sup>fghij</sup>	28.3 <sup>fghij</sup>	23.8 <sup>i</sup>	24.6 <sup>ij</sup>
Untreated rice hulls	31.2 <sup>defg</sup>	24.6 <sup>ij</sup>	28.9 <sup>fghij</sup>	24.3 <sup>j</sup>	24.5 <sup>ij</sup>
Commercial formaldehyde-treated rice hulls (2 kg/ton) <sup>2</sup>	30.3 <sup>defgh</sup>	24.2 <sup>j</sup>	28.5 <sup>fghij</sup>	23.7 <sup>i</sup>	24.5 <sup>ij</sup>
Concentrated commercial formaldehyde <sup>2</sup>	36.7 <sup>bc</sup>	45.0 <sup>a</sup>	43.0 <sup>a</sup>	45.0 <sup>a</sup>	45.0 <sup>a</sup>
Concentrated dry commercial benzoic acid and probiotic blend <sup>3</sup>	30.6 <sup>defgh</sup>	26.1 <sup>ghij</sup>	29.8 <sup>efghi</sup>	26.4 <sup>fghij</sup>	26.3 <sup>ghij</sup>
Ready-to-use liquid commercial food-grade sanitizer <sup>4</sup>	27.9 <sup>fghij</sup>	24.9 <sup>ij</sup>	28.3 <sup>fghij</sup>	24.7 <sup>ij</sup>	26.0 <sup>ghij</sup>
3% dilution of liquid hydrogen peroxide commercial product <sup>5</sup>	27.7 <sup>fghij</sup>	25.4 <sup>hij</sup>	27.8 <sup>fghij</sup>	24.7 <sup>ij</sup>	27.2 <sup>fghij</sup>
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product <sup>6</sup>	31.7 <sup>cdef</sup>	27.1 <sup>fghij</sup>	29.7 <sup>efghi</sup>	26.3 <sup>ghij</sup>	27.3 <sup>fghij</sup>
10% dilution of liquid sodium hypochlorite commercial sanitizer <sup>7</sup>	25.4 <sup>hij</sup>	35.0 <sup>bcde</sup>	43.0 <sup>a</sup>	35.6 <sup>bcd</sup>	37.1 <sup>b</sup>
Concentrated liquid medium chain fatty acid blend <sup>8</sup>	31.1 <sup>defg</sup>	26.3 <sup>ghij</sup>	27.4 <sup>fghij</sup>	26.0 <sup>ghij</sup>	26.0 <sup>ghij</sup>
<i>P</i> =	0.001				
SEM	1.91				

<sup>1</sup> This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.

<sup>2</sup> Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.

<sup>3</sup> VevoVital and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.

<sup>4</sup> DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride.

<sup>5</sup> INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide.

<sup>6</sup> Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde.

<sup>7</sup> Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite.

<sup>8</sup> Caprylic, caproic and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.

<sup>abcde fghijkl</sup> Means with different superscripts differ (*P* < 0.05).

## Evaluating the Impact of VevoVitall and/or CRINA as Potential Porcine Epidemic Diarrhea Virus Mitigation Strategies as Determined by Polymerase Chain Reaction Analysis and Bioassay<sup>1,2</sup>

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### Summary

Feed and feed ingredients have been shown to be potential vectors of porcine epidemic diarrhea virus (PEDV). Potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed milling industries. Therefore, the objective of this experiment was to determine the impact of VevoVitall (5,000 ppm; DSM Nutritional Products Inc., Parsippany, NJ), CRINA (200 ppm; DSM Nutritional Products Inc., Parsippany, NJ), and a combination of both products (COMBINATION; 5,000 ppm VevoVitall and 200 ppm CRINA) as feed additives with potential to mitigate the risk of PEDV, in swine gestation diet (FEED) and spray-dried porcine plasma (SDPP) as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analyzed at seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42) and bioassay. There was a marginally significant treatment × feed matrix × day interaction ( $P = 0.082$ ), in which the cycle threshold (Ct) value increased over time in the diet when treated with the COMBINATION, whereas, there was no increase over time observed in SDPP. There was a highly significant ( $P < 0.001$ ) feed matrix × day interaction in which the Ct increased over time in FEED, whereas, there was very little increase over time observed in SDPP.

<sup>1</sup> Appreciation is expressed to Dr. Charles Farenholtz (Phibro Animal Health, Teaneck, NJ) for technical support and use of facilities and equipment, Dr. Dick Hesse and Joe Anderson for technical support and laboratory use, Elizabeth Poulsen and Rusty Ransbrough for technical support and laboratory use, Dr. Joe Crenshaw (APC Functional Proteins, Ankeny, IA), as well as Marut Saensukjaroenphon and Mary Muckey for technical support.

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Additionally, there was a marginally significant treatment  $\times$  feed matrix interaction ( $P = 0.079$ ). Overall, the COMBINATION was most effective at reducing the quantity of genetic material as detected by qRT-PCR ( $P < 0.001$ ). Virus shedding was observed in the d 7 post-inoculation SDPP COMBINATION treatment, as well as d 0 FEED COMBINATION treatment. No other treatment bioassay room had detectable RNA shed and detected in fecal swabs or cecal contents (d 1, 3, 7, 14, and 21 post-laboratory inoculation FEED, COMBINATION).

In summary, the combination of CRINA and VevoVital enhanced degradation of PEDV RNA in swine feed, but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of CRINA and VevoVital caused infection at d 0 post-laboratory inoculation; however, neither set of samples was infective at d 1 post-laboratory inoculation.

Key words: feed additive, feed matrix, PEDV, swine

## Introduction

Feed and feed ingredients have been shown to be potential vectors of Porcine Epidemic Diarrhea virus (PEDV).<sup>7,8</sup> Therefore, potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed manufacturing industries. Research has been conducted assessing potential mitigation techniques, such as the use of certain feed additives or thermal processing during pelleting of diets. During the pelleting of complete swine diets, previous research has shown that a pelleting conditioner temperature of 130°F was effective at minimizing the risk of PEDV transfer.<sup>9</sup> The application of certain feed additives, including medium chain fatty acids, essential oils, organic acids, and formaldehyde, has been effective at degrading PEDV genetic material in complete feed and feed ingredients as quantified by quantitative real-time polymerase chain reaction (qRT-PCR), but lack of infectivity has not been verified via bioassay.<sup>10</sup>

CRINA and VevoVital are two commercially available products sold by DSM Nutritional Products (Parsipanny, NJ). CRINA is a combination of essential oils designed to stimulate gut health in swine, and VevoVital is a 99.9% benzoic acid product designed

<sup>7</sup> Dee, S., T. Clement, A. Schelkopf, J. Nerem, D. Knudsen, J. Christopher-Hennings, and E. Nelson. 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naive pigs following consumption via natural feeding behavior: Proof of concept. *BMC Veterinary Research*. 10(176).

<sup>8</sup> Pillatzki, A. E., P. C. Gauger, D. M. Madson, E. R. Burrough, JianQiang Zhang, Q. Chen, D. R. Magstadt, P. H. E. Arruda, G. W. Stevenson, and K. J. J. Yoon. 2015. Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. *Journal of Swine Health and Production*. 23(6): 317-320.

<sup>9</sup> Cochrane, R. A., S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, R. A. Hesse, JianQiang Zhang, M. D. Tokach, J. Bai, and C. K. Jones. 2015. Evaluating chemical mitigation of Porcine Epidemic Diarrhea Virus (PEDV) in swine feed and ingredients. *Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports*. Vol. 1: Iss. 7.

<sup>10</sup> Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, JianQiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of thermal mitigation on Porcine Epidemic Diarrhea Virus (PEDV)-contaminated feed. *Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports*. Vol. 1: Iss. 7.

to reduce activity of microorganisms in feed, including fungi, yeasts and certain bacteria, such as *E. coli* and *Salmonella*.<sup>11</sup> However, neither CRINA nor VevoVitall have been tested as potential PEDV mitigants. Therefore, the objective of this experiment was to determine the impact of VevoVitall and CRINA as feed additives with the potential to mitigate PEDV contamination of feed and spray-dried porcine plasma as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bioassay.

## Procedures

Treatment structure was designed in a  $2 \times 2 \times 2$  factorial arrangement with two feed matrices (FEED and SDPP) and feed additive treatment factors including VevoVitall (5,000 ppm; DSM Nutritional Products Inc., Parsippany, NJ) and CRINA (200 ppm; DSM Nutritional Products Inc., Parsippany, NJ), and combination of both products (COMBINATION; 5,000 ppm VevoVitall and 200 ppm CRINA). The swine diet (Table 1) used in this experiment was manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and was verified to be devoid of PEDV and porcine delta-coronavirus (PDCoV) ribonucleic acid (RNA) as determined via qRT-PCR prior to initiation of the experiment. Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA) was also verified to be free of both PEDV and delta-coronavirus RNA prior to use as verified by qRT-PCR.

### *Feed Additive Treatment*

Prior to treatment of feed matrices with feed additive treatments, a 25.0 g sample of each feed matrix was collected and placed in its appropriate bottle. These samples received no virus, and were the positive control samples reserved for the bioassay portion of the experiment. A benchtop paddle mixer was used as previously described<sup>12</sup> for mixing dry products with FEED. Mixing time was 3 min, as was previously verified with a CV of < 10% using a chloride mixer efficiency procedure (Quantab; Hach Co., Loveland, CO). A V-mixer (Cross-Flow Blender; Patterson-Kelley Co., East Stroudsburg, PA) was used to mix feed additive treatments with SDPP. A mixer efficiency test was performed using spray-dried bovine plasma, and resulted in a uniform mix with a mix time of 7.0 min (MicroTracer-F; Microtracers Inc., San Francisco, CA).

Following the mixing of feed matrix and corresponding feed additive treatment, 22.5 g of chemically treated feed matrix was sampled and placed in the appropriate bottle (250 mL Nalgene square wide-mouth HDPE; Thermo Fisher Scientific, Waltham, MA) to be inoculated with PEDV and analyzed on seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42), with 3 replications of each sampling day/feed additive treatment combination. This process was repeated for each feed matrix  $\times$  feed additive treatment combination. Both the paddle mixer and V-blender were cleaned between feed additive treatments initially by high-pressure air, then a flush step was performed

<sup>11</sup> DSM Nutritional Products, Inc., Parsippany, NJ; [http://www.dsm.com/markets/anh/en\\_US/products/products-eubiotics/products-eubiotics-vevovital.html](http://www.dsm.com/markets/anh/en_US/products/products-eubiotics/products-eubiotics-vevovital.html), Accessed 1/13/2016.

<sup>12</sup> Schumacher, L. L., J. C. Woodworth, C. R. Stark, C. K. Jones, R. A. Hesse, R. G. Main, Jianqiang Zhang, P. C. Gauger, S. S. Dritz, and M. D. Tokach. 2015. Determining the minimum infectious dose of Porcine Epidemic Diarrhea Virus (PEDV) in a feed matrix. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

with either untreated FEED or SDPP for the paddle mixer and V-blender, respectively, followed by a final cleaning with high-pressure air.

### *Inoculation*

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

### *Real-time PCR analysis*

Separate bottles were analyzed on d 0, 1, 3, 7, 14, 21, and 42 post-laboratory inoculation. On each day of analysis, 100 mL phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) was added to each bottle predetermined for analysis on that day. Bottles were shaken for approximately 10 sec, at which point they were allowed to settle overnight at 39.2°F. The following day, supernatant was pulled and aliquoted for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at -4°F until the conclusion of the trial, at which point qRT-PCR analysis was performed on one aliquot per sample bottle and the remaining three samples per bottle were stored at -112°F until transported to Iowa State University for the initiation of the bioassay portion of the experiment.

After collection of d 42 post-laboratory inoculation aliquots, qRT-PCR was conducted on designated preserved aliquots at Kansas State University Veterinary Diagnostic Laboratory Molecular Diagnostics Lab as previously described.<sup>12</sup> Fifty microliters ( $\mu$ L) of supernatant from each sample was loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburg, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions with one modification, reducing the final elution volume to 60  $\mu$ L. One negative extraction control consisting of all reagents except the sample was included in each extraction. The extracted RNA was frozen at -4°F until assayed by qRT-PCR. Analyzed values represent cycle threshold (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until viral genetic material is detected, thus lower quantities of genetic material are present in the original sample.

### *Bioassay*

A bioassay was performed using selected treatment × time combinations at Iowa State University Veterinary Diagnostic Laboratory to determine the viral infectivity characteristics following protocols previously described by Pillatzki et al., 2015<sup>13</sup> and Thomas et al., 2015.<sup>14</sup>

The experimental protocol for the pig bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee. Forty-eight crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Upon arrival, piglets were ear tagged, weighed, and administered a dose of cefitiofur (Excede, Zoetis, Florham Park, NJ). Also upon arrival, fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) using a qRT-PCR assay. To further confirm PEDV negative status, serum was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and TGEV antibody by enzyme-linked immunosorbent assay (ELISA) conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began.

Briefly, pigs from each experimental treatment were housed in separate rooms with independent ventilation systems. Rooms had solid flooring that was minimally rinsed to reduce risk of PEDV aerosolization. Pigs were fed liquid milk replacer twice daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each pig was administered 10 mL of the PBS supernatant treatment by orogastric gavage using an 8-gauge French catheter 0 d post-bioassay inoculation (dpi).

Rectal swabs were collected on d -2, 0, 2, 4, and 6 dpi from all piglets and tested for PEDV RNA via qRT-PCR. Fresh small intestine, cecum, and colon were collected at necropsy at 7 dpi, along with an aliquot of cecal content. One section of formalin-fixed proximal, middle, and distal jejunum and ileum were collected for histopathology. Cecal content was evaluated for PEDV via qRT-PCR. Tissue was routinely processed and fixed in neutral buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin stain. One section of proximal, middle, and distal jejunum; and three serial sections from the piece of ileum (for a total of six sections of intestine) were evaluated by a veterinary pathologist blind to the treatments. Morphology and IHC data were excluded from the current report.

<sup>13</sup> Pillatzki, A. E., P. C. Gauger, D. M. Madson, E. R. Burrough, Zhang JianQiang, Q. Chen, D. R. Magstadt, P. H. E. Arruda, G. W. Stevenson, and K. J. J. Yoon. 2015. Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. *Journal of Swine Health and Production*. 23(6): 317-320.

<sup>14</sup> Thomas, J. T., Qi Chen, P. C. Gauger, L. G. Gimenez-Lirola, Avanti Sinha, K. M. Harmon, D. M. Madson, E. R. Burrough, D. R. Magstadt, H. M. Salzbrenner, M. W. Welch, Yoon Kyoung-Jin, J. J. Zimmerman, and Zhang Jian Qiang. 2015. Effect of porcine epidemic diarrhea virus infectious doses on infection outcomes in naive conventional neonatal and weaned pigs. *PLOS ONE*. 10(10): e0139266.

### *Statistical Analysis*

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine the main effects of feed additive treatment, feed matrix, as well as day post-laboratory inoculation and all associated interactions on PEDV Ct values with individual sample bottle as the experimental unit. Bottle within treatment was included in the model as the subject of the repeated measure of day after laboratory inoculation. Bottle was included in the statistical model as a random effect. Results for the response criteria were considered significant at  $P \leq 0.05$  and marginally significant from  $P > 0.05$  to  $P \leq 0.10$ .

## **Results and Discussion**

### *Quantity of Detectable Viral RNA*

There was a marginally significant treatment  $\times$  feed matrix  $\times$  day interaction ( $P = 0.082$ , Table 2) in which the combination of CRINA and VevoVital resulted in a reduction of quantifiable RNA on d 21 and 42 at a greater rate in feed than in the SDPP matrix. There was a significant ( $P < 0.001$ , Table 2) feed matrix  $\times$  day interaction in which the Ct value increased over time in gestation diet, whereas there was very little increase over time observed in SDPP. Additionally, there was a marginally significant treatment  $\times$  feed matrix interaction ( $P = 0.079$ , Table 3) in which the combination of CRINA and VevoVital was more effective at reducing the amount of quantifiable RNA in FEED relative to no feed additive treatment or feed additives included individually, and was no different than untreated or treatment with CRINA or VevoVital individually in the SDPP matrix. There was no treatment  $\times$  day interaction ( $P = 0.234$ ). All main effects were highly significant, including treatment, day, and feed matrix ( $P \leq 0.003$ , Tables 2 and 3). Overall, the combination of CRINA and VevoVital was most effective at reducing the quantity of genetic material ( $P < 0.001$ , Ct = 33.0; Table 3), regardless of feed matrix or day post-inoculation. All three feed samples treated with the COMBINATION did not have detectable PEDV RNA at d 42 post-laboratory inoculation, and two samples did not have detectable virus at d 21 post-laboratory inoculation. Cochrane et al. (2015) observed increased efficacy at reducing the amount of quantifiable RNA in complete swine diet and blood meal as the duration of the study progressed using a 2% essential oil blend (garlic oleoresin, turmeric oleoresin, capsicum oleoresin, rosemary extract, and wild oregano essential oils). The maximum efficacy was 14 d post-inoculation and beyond in blood meal and beyond 21 d post-inoculation in the complete swine diet. In the current study, there was no difference in quantification of genetic material among the untreated control, CRINA, and VevoVital treatments ( $P > 0.10$ ; Ct = 31.8, 31.8, 31.9, respectively). Overall, a greater quantity of PEDV RNA was detected in SDPP relative to feed ( $P < 0.001$ , Ct =  $29.3 \pm 0.28$  vs.  $35.0 \pm 0.28$ , SDPP vs. feed, respectively). The PEDV Ct increased between d 0, 1, 3, 21, and 42 post-laboratory inoculation ( $P < 0.001$ ; 29.3, 30.7, 31.6, 33.9, and 35.2, respectively). There was no difference in Ct between d 3, 7, and 14 post-laboratory inoculation ( $P > 0.05$ , 31.6, 32.1, and 32.2, respectively).

### *Infectivity*

Upon completion of PCR testing, sixteen samples were strategically selected for assessment of virus infectivity via a bioassay at Iowa State University. The samples selected were d 0 negative control, 7 positive control, and 7 combination of CRINA

and VevoVitall samples. Each sample consisted of 3 supernatant aliquots that each were gavaged into a single pig within bioassay room. Six combinations were selected using swine feed and the combination of CRINA and VevoVitall (d 0, 1, 3, 7, 14, and 21 post-laboratory inoculation) and an additional set of samples was selected using the combination of CRINA and VevoVitall 7 d after inoculation in SDPP. Positive control samples included untreated FEED and SDPP samples at d 0, 3, and 21 post-laboratory inoculation as well as d 1 FEED positive control for a total of 7 total positive control bioassay rooms. The d 0 and d 1 FEED positive control samples were from the current study, however the other 5 positive control samples were in conjunction with additional research from our laboratory using identical procedures in which bioassay controls were shared across projects (Ct = 29.4, 34.1, 31.6, 37.3, 37.8; d 0 SDPP, d 3 FEED, d 3 SDPP, d 21 FEED, d 21 SDPP, respectively).

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

The d 0 FEED combination of CRINA and VevoVitall pigs (3/3) were shedding PEDV RNA as detected by fecal swabs beginning on 2 dpi and remained infected through necropsy at 7 dpi. Virus shedding was observed in fecal swabs in one d 7 post-bioassay inoculation SDPP COMBINATION pig 2 dpi, and all three pigs were shedding virus at 6 dpi and had virus detectable in cecal contents at necropsy. None of the COMBINATION treated FEED had detectable RNA in fecal swabs or cecal contents with the exception of d 0 post-laboratory inoculation samples.

In summary, the combination of CRINA and VevoVitall enhanced degradation of PEDV RNA in swine feed, but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of CRINA and VevoVitall resulted in PEDV infection at d 0 post-laboratory inoculation; however, neither set of samples were infective at d 1 post-laboratory inoculation.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

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

<sup>1</sup> Each kilogram contains 26.4 g Mn, 110 g Fe, 110 g Zn, 11 g Cu, 198 mg I, and 198 mg Se.

<sup>2</sup> Each kilogram contains 110,000 mg choline, 44 mg biotin, 330 mg folic acid, 990 mg pyridoxine.

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

<sup>4</sup> HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

**Table 2. Interactive means of VevoVitall and/or CRINA, matrix, and day, matrix by day interaction, and main effect of day on PEDV detection as determined by qRT-PCR<sup>1</sup>**

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

<sup>1</sup> An initial tissue culture (2.5 mL diluted virus inoculum, 10<sup>5</sup> TCID<sub>50</sub>/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVitall, combination of CRINA and VevoVitall (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. Values are represented by mean quantified PEDV RNA cycle threshold (Ct) value as determined by qRT-PCR.

<sup>(x/x)</sup> Superscripts denote number of samples with cycle threshold for PEDV RNA below detectable limit of Ct = 45. A value of 45.0 was used for assumed for samples with non-detectable RNA for analysis.

<sup>2</sup> Matrix × treatment × day interaction, *n* = 3 for each value. SEM = 0.90 cycle threshold, *P* = 0.082.

<sup>3</sup> Matrix × day interaction, *n* = 12 for each value. SEM = 0.50 cycle threshold, *P* < 0.001.

<sup>4</sup> Main effect of day, *n* = 24 for each value. SEM = 0.38 cycle threshold, *P* < 0.001.

<sup>abdef</sup> Means within interaction or effect lacking a common superscript differ (*P* < 0.05).

**Table 3. Interactive means of feed matrix and treatment, and main effect of treatment on PEDV detection using qRT-PCR<sup>1,2</sup>**

Item	Control	CRINA	VevoVitall	CRINA + VevoVitall	SEM	<i>P</i> =
Matrix × treatment						
FEED <sup>3</sup>	34.5 <sup>b</sup>	34.5 <sup>b</sup>	34.6 <sup>b</sup>	36.5 <sup>a</sup>	0.37	0.079
SDPP <sup>4</sup>	29.1 <sup>c</sup>	29.1 <sup>c</sup>	29.2 <sup>c</sup>	29.6 <sup>c</sup>		
Treatment	31.8 <sup>b</sup>	31.8 <sup>b</sup>	31.9 <sup>b</sup>	33.0 <sup>a</sup>	0.28	0.003

<sup>1</sup> An initial tissue culture (2.5 mL diluted virus inoculum, 10<sup>5</sup> TCID<sub>50</sub>/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVitall, combination of CRINA and VevoVitall (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. A total of 168 samples were used for the analysis with each treatment represented by a mean of *n* = 21 for the matrix × treatment interaction, and *n* = 42 for the main effect of treatment.

<sup>2</sup> Cycle threshold required to detect genetic material. A higher Ct value is indicative of less genetic material present.

<sup>3</sup> Swine gestation diet.

<sup>4</sup> Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA).

<sup>abc</sup> Means within interaction or effect lacking common superscript differ (*P* < 0.05).

**Table 4. Effects of VevoVital and/or CRINA as potential porcine epidemic diarrhea virus (PEDV) mitigation strategies on PEDV detection from feed, pig fecal swabs, and cecum contents<sup>1</sup>**

Item	Fecal swabs <sup>2</sup>				Cecum contents
	-2 dpi	2 dpi	4 dpi	6 dpi	7 dpi
<b>FEED</b>					
No treatment					
d 0 no virus	---	---	---	---	---
d 0	---	+++	+++	+++	+++
d 1	---	---	---	---	---
d 3	---	---	---	---	---
d 21	---	---	---	---	---
CRINA + VevoVital					
d 0	---	+++	+++	+++	+++
d 1	---	---	---	---	---
d 3	---	---	---	---	---
d 7	---	---	---	---	---
d 14	---	---	---	---	---
d 21	---	---	---	---	---
<b>SDPP</b>					
No treatment					
d 0 no virus	---	---	---	---	---
d 0	---	+++	+++	+++	+++
d 3	---	+++	+++	+++	+++
d 21	---	---	---	---	---
CRINA + VevoVital					
d 7	---	+-	++-	+++	+++

<sup>1</sup>An initial tissue culture 2.5 mL diluted virus inoculum, 10<sup>5</sup> TCID<sub>50</sub>/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVital, combination of CRINA and VevoVital (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. The supernatant from each sample was then collected for pig bioassay on the appropriate day post-laboratory inoculation and preserved until initiation of the bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Pigs were initially 10 d old initial BW = 7.9 lb.

<sup>2</sup>Day post-bioassay inoculation.

## Evaluation of the Effects of Flushing Feed Manufacturing Equipment with Chemically-Treated Rice Hulls on Porcine Epidemic Diarrhea Virus Cross Contamination During Feed Manufacturing<sup>1,2</sup>

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### Summary

Various strategies have been proposed to mitigate potential risk of porcine epidemic diarrhea virus (PEDV) transmission via feed and feed ingredients. Wet decontamination has been found to be the most effective decontamination of feed mill surfaces; however, this is not practical on a commercial feed production-scale. Another potential mitigation strategy, easier to implement, would be using chemically-treated rice hulls flushed through the feed manufacturing equipment. The objective of this experiment was to determine the impact of MCFA- or formaldehyde-treated rice hull flush batches as potential PEDV mitigation strategies during feed manufacturing. Feed without evidence of PEDV RNA contamination was inoculated with PEDV. Based on PCR analysis, this feed had a Ct = 30.2 and was confirmed infective in bioassay. After manufacture of PEDV positive feed, untreated rice hulls, or rice hulls treated with Sal CURB, 2%, or 10% medium chain fatty acid blend (MCFA; 1:1:1 ratio of caproic, caprylic, and capric acid) were flushed through laboratory-scale mixers. For the untreated rice hulls, 3 of 6 samples had detectable PEDV RNA (avg. Ct = 41.4) while 1 of 6 Sal CURB treated rice hull flush samples and 2 of 6 of the 2% MCFA rice hull flush samples had detectable PEDV RNA. However, PEDV RNA was not detected in any of the 10% MCFA rice hull flush samples. Additionally, rice hulls treated with 10% MCFA were mixed and discharged through a production-scale mixer and bucket elevator following manu-

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facturing of PEDV positive feed. In the production-scale system, no rice hull flush or feed samples from the mixer following chemically-treated rice hull flush had detectable PEDV RNA. However, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectable PEDV RNA.

Dust collected following mixing of PEDV-contaminated feed had a large quantity of PEDV RNA (avg. Ct = 29.4). Dust collected immediately after the 10% MCFA rice hull flush batch had a reduced quantity of PEDV RNA (Ct = 33.7), and the subsequent feed following the 10% rice hull flush had no detectable PEDV RNA. Pigs inoculated with dust collected after manufacturing PEDV-positive feed were shedding PEDV RNA by 2 dpi and continued to have detectable RNA until necropsy. Dust collected from the 10% MCFA rice hull flush batch or the subsequent batch was not infective.

Overall, the use of rice hull flushes effectively reduced the quantity of detectable RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with Sal CURB and 10% MCFA provided additional reduction in detectable RNA present in the rice hull flush samples. Finally, dust collected after manufacturing PEDV-inoculated feed contains a very high quantity of viral RNA and was found infective, demonstrating it has the potential to serve as a vector for PEDV transmission.

Key words: chemical treatment, flush, medium chain fatty acid, PEDV, swine

## Introduction

Feed manufacturing equipment has been shown to be a potential source of porcine epidemic diarrhea virus (PEDV) cross contamination.<sup>6,7</sup> Wet decontamination has been found to be the most effective method for decontaminating the surface of feed mill equipment. However, this is not practical in most current commercial feed production settings. Methods to mitigate the risk of PEDV transmission in feed and feed ingredients have been investigated, including chemical mitigation using products such as formaldehyde, medium chain fatty acids, essential oils, or dietary acidifiers, as well as thermal mitigation accomplished by pelleting diets.<sup>8,9</sup> These methods are not universally

<sup>6</sup> Schumacher, L. L., R. A. Cochrane, C. E. Evans, J. R. Kalivoda, J. C. Woodworth, C. R. Stark, C. K. Jones, R. G. Main, Jianqiang Zhang, S. S. Dritz, P. C. and Gauger. 2015. Evaluating the Effect of Manufacturing Porcine Epidemic Diarrhea Virus (PEDV)-Contaminated Feed on Subsequent Feed Mill Environmental Surface Contamination. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

<sup>7</sup> Schumacher, L. L., R. A. Cochrane, J. C. Woodworth, C. R. Stark, C. K. Jones, R. G. Main, J. Zhang, P. C. Gauger, S. S. Dritz, and M. D. Tokach. 2015. Utilizing Feed Sequencing to Decrease the Risk of Porcine Epidemic Diarrhea Virus (PEDV) Cross-contamination During Feed Manufacturing. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

<sup>8</sup> Cochrane, R. A., S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, R. A. Hesse, J. Zhang, M. D. Tokach, J. Bai, and C. K. Jones. 2015. Evaluating Chemical Mitigation of Porcine Epidemic Diarrhea Virus (PEDV) in Swine Feed and Ingredients. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

<sup>9</sup> Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV) Contaminated Feed. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

applicable to all feed manufacturing facilities due to equipment cost and/or lack of application equipment.

Another key deterrent from using chemical mitigation strategies in complete swine diets is the economic burden from including products in all feed that is produced. Other research has assessed sequencing batches of PEDV-negative feed following an inoculated batch of feed to assess the effectiveness of reducing the risk of viral transmission.<sup>7</sup> A series of diets intended for low-risk cohorts of livestock would be manufactured to essentially “flush” the system prior to manufacturing diets intended for high-risk groups, such as breeding stock. While this may be the most practical mitigation technique for feed mills to implement, there still remains a significant quantity of viral particles on feed-contact surfaces, and environmental contamination, including dust production and distribution throughout the facility. This dust may pose a risk for contamination of subsequent diets. One potential solution is to use chemical mitigants as a periodic flush step within the feed manufacturing process. The periodic nature would reduce the cost and may be an easily implementable, more cost-effective mitigation strategy than using chemical mitigants in each batch. Rice hulls were selected as the carrier for this chemical flush because the ingredient is commonly used as a carrier for vitamins and other micro-nutrients due to its relatively low cost. Furthermore, it has a high degree of abrasiveness, which may help facilitate the removal of viral contamination on equipment surfaces. Therefore, the objective of this experiment was to determine the impact of MCFA- or formaldehyde-treated rice hull flush batches as potential PEDV mitigation strategies during feed manufacturing.

## Procedures

### *General*

The experiment was conducted at the Kansas State University Cargill Feed Safety Research Center (FSRC). Prior to the experiment, the FSRC was decontaminated following a standard protocol approved by the Kansas State University Institutional Biosafety Committee. Prior to initiation of the experiment, the FSRC was physically cleaned using compressed air and sweeping, chemically cleaned using a two-step process using a 1:256 dilution of ammonium glutaraldehyde blend (Synergize; Preserve International, Reno, NV) and a 1:32 dilution of sodium hypochlorite solution. The facility was then heated to 140°F for a minimum of 24 h and cooled to room temperature at which point the environmental surfaces were sampled (World Bioproducts, Mundelein, IL) and verified devoid of PEDV viral RNA to ensure efficacy of the disinfection procedures prior to initiation of the experiment. After chemical disinfection, the facility was held in containment mode with negative air pressure and High-Efficiency Particulate Arrestance (HEPA) filters preventing contaminated air from leaving the facility. Containment was maintained throughout the experiment and through the post-decontamination procedures using the same procedures described above.

The swine diet (Table 1) used in this experiment was manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and was verified to be devoid of PEDV and porcine delta-coronavirus (PDCoV), viral RNA as determined via qRT-PCR prior to initiation of the experiment. Rice hulls were also analyzed using qRT-PCR prior to the experiment to confirm lack of PEDV genetic material. The production-scale mixer used was a 0.14-yd<sup>3</sup> electric paddle mixer (H.C.

Davis Sons Manufacturing, model # SS-L1; Bonner Springs, KS) with a mix time of 5 min. Feed was discharged at a rate of approximately 10 lb/min into a bucket elevator (Universal Industries, Cedar Falls, IA) fitted with 74 buckets (114 cm<sup>3</sup> each), and then discharged through a 10 inch diameter discharge spout, and collected in plastic biohazard bags. Laboratory-scale stainless steel paddle mixers ( $n = 13$ ; Cabela's Inc., Sidney, NE) were validated for mixer efficiency for 5.5 and 11.0 lb batches using a mix time of 5 min. Validation of mixers prior to the experiment to achieve a coefficient of variation of less than 10% was done following previously described procedures.<sup>9</sup>

### ***Chemical Treatment of Flush Batches and Negative Samples***

Prior to initiation of the experiment, six 5.5 lb chemically-treated rice hull batches were prepared using 2% MCFA blend ( $n = 2$ ; 1:1:1 ratio of caproic, caprylic, and capric acid), 10% MCFA blend ( $n = 2$ ; same ratio of acids used as in 2% blend), or commercial formaldehyde ( $n = 2$ ; Sal CURB, Kemin Industries, Inc.; application rate = 6.5 lb/T). Untreated rice hulls (5.5 lb;  $n = 2$ ) were also weighed and prepared prior to initiation of the experiment. Rice hulls (untreated and chemically-treated) were stored in double lined bags for 48 hours at room temperature (70°F) until initiation of experiment.

Prior to inoculation with PEDV, batches of feed were placed in, mixed, and discharged through both a laboratory-scale mixer and production-scale systems. For the laboratory-scale mixers, 500 g of PEDV negative feed was added to each mixer, rotated for approximately 15 sec, then disconnected from the drive unit and inverted in a one-step motion to dispose of feed into a waste container. A small quantity of residual feed remained in each mixer after this systematic priming discharge procedure. Following the priming of each mixer, a 5.5 lb batch of PEDV-negative feed was added to each laboratory-scale mixer and mixed as described above. The mixer then was shut off, drive coupler removed from the drive unit motor, and a subsample was collected from six locations within each mixer for a total sample size of approximately 0.5 lb. The mixer was then fully disconnected and inverted to dispose of feed into a waste container.

After priming and collection of the negative feed sample from laboratory-scale mixer, the production-scale system was primed and negative sample collected. An 11 lb batch of PEDV-negative feed was added to the production-scale mixer, allowed to mix for approximately 15 seconds, and subsequently discharged into the bucket elevator and was collected at the discharge spout to prime the mixer and fill the boot of the bucket elevator. A 110 lb batch of PEDV-negative feed was then added to the production-scale mixer, mixed for 5 min, and then discharged into the bucket elevator and collected in bags at the discharge spout. A sample of feed was collected from multiple subsample points within the discharged batch of feed.

### ***Laboratory-Scale Mixer Inoculation, Flush, and Subsequent Feed***

The viral inoculum was cell culture derived (USA/IN/2013/19338, passage 9) and had an initial concentration of  $4 \times 10^6$  TCID<sub>50</sub>/mL. A 1:10 dilution was performed using phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) to create 2,500 mL of  $10^5$  TCID<sub>50</sub>/mL viral inoculum. Inoculation of feed to be used in each of the laboratory-scale mixers was performed in 11.0 lb batches using an additional laboratory-scale mixer in which 9.9 lb of PEDV negative feed was added to the mixer and 500 mL of  $10^5$  TCID<sub>50</sub>/mL diluted viral inoculum was added to create 11.0 lb of

$10^4$  TCID<sub>50</sub>/g inoculated feed. This batch was mixed for 5 min, at which point it was split into two samples using a riffle splitter and weighed into 5.5 lb batches, bagged, and stored in a freezer (10°F) until inoculated into appropriate laboratory-scale mixer. This process was repeated three additional times, to create a total of eight 5.5 lb batches of inoculated feed.

After preparation of laboratory-scale mixer inoculated feed, each of 8 laboratory-scale mixers was inoculated with feed, flush step performed, and a subsequent batch of feed was mixed and sampled. For each inoculation, a bagged sample of PEDV-inoculated feed was randomly selected from the freezer and placed into the randomly selected laboratory-scale mixer. Feed was mixed for 5 min, at which point a sample of PEDV-inoculated feed was collected from 6 locations within the mixer. PEDV-inoculated feed was then discarded into biohazard waste bags using a complete inversion of the mixer following the systematic procedure as described above with no tapping or additional cleaning action. The appropriate flush batch was added to the mixer and mixed for 5 min. A sample of the rice hull flush was collected from 6 locations within the mixer as previously described. The remaining flush was then discarded, and a subsequent 5.5 lb batch of PEDV-negative feed was added to the mixer and mixed. After mixing, a sample of the subsequent feed was collected, and remaining feed was discarded. This process was repeated 7 additional times in a random order blocked by repetition number, for a total 8 laboratory-scale mixers with two replicates of each of the four chemical treatments (untreated rice hulls, Sal CURB-treated rice hulls, 2% MCFA-treated rice hulls, and 10% MCFA-treated rice hulls).

### ***Production-Scale System Inoculation, Flush, and Subsequent Feed***

For inoculation of the production-scale system, a 9.9 lb batch of PEDV-negative feed was added to a clean laboratory-scale paddle mixer and 500 mL of  $10^6$  TCID<sub>50</sub>/mL inoculum was slowly added to create an 11 lb batch of PEDV inoculated feed ( $10^5$  TCID<sub>50</sub>/g). Upon conclusion of the addition of the virus, the batch was mixed for 5 min to ensure an even mix of virus into the feed inoculum. The PEDV feed inoculum was then added to 99 lb of PEDV-free swine diet in the production-scale mixer to create the 110-lb batch of PEDV positive feed ( $10^4$  TCID<sub>50</sub>/g). The entire batch of PEDV-positive feed was then mixed for 5 min, discharged into the bucket elevator, and collected at the bucket elevator discharge spout in biohazard waste bags. A sample of PEDV-positive feed was collected from multiple locations within the discharged batch of PEDV-positive feed. This sample of PEDV inoculated feed was combined at a 1:1 ratio with PEDV-inoculated feed (also  $10^4$  TCID<sub>50</sub>/g) from laboratory-scale mixer to create a single PEDV-positive sample.

After inoculation of the production-scale mixer, 79.2 lb of ground rice hulls were added directly to the mixer, along with 8.8 lb of MCFA (1:1:1 ratio of caproic, caprylic, and capric acid) to create a 10% MCFA rice hull flush with a similar mixer fill volume as a 110 lb batch of feed. After a 5 min mix time, 6 samples were collected from various locations within the mixer. The rice hull flush batch was then discharged into the bucket elevator and collected at the bucket elevator discharge spout. Samples of discharged flush material were collected at multiple times during discharge to create a single composite sample.

A 110 lb batch of PEDV-negative feed was then added to the production-scale mixer and allowed to mix for 5 min. A 0.5 lb sample was collected from the mixer and remaining feed was discharged into the bucket elevator and collected at the bucket elevator discharge spout. Another 0.5 lb sample was collected from six locations of the bucket elevator to create a single composite sample. Samples were placed on ice and transported to the laboratory for qRT-PCR analysis preparation.

Dust samples were also collected throughout the experiment, including dust collected after mixing of  $10^4$  TCID<sub>50</sub>/g inoculated feed in both the laboratory and production-scale systems, after mixing of 10% MCFA treated rice hulls in the production-scale mixer, and collected from mixing of the subsequent feed following the 10% MCFA rice hull flush. All dust collection surfaces were above the fill level of the mixer; therefore, all collected dust had become airborne before depositing on the collection surfaces. Dust was collected from the same surface after each batch of feed (positive inoculated feed, 10% MCFA rice hull flush, and subsequent PEDV-free feed); therefore, dust collected was produced during the associated mixing process and not from previous manufacturing processes.

### *Viral RNA Quantification*

After sample collection, temporary storage on ice, and transport to the Kansas State University Molecular Diagnostic Research and Development Laboratory, three 50.0 g subsamples of feed from each collection point were added to individual 500 mL high density polyurethane (HDPE) bottles. Rice hull samples from each collection point were subsampled into three 25.0 g samples and added to individual 250 mL HDPE bottles. After subsampling of all feed and rice hull flush samples into appropriate bottles, varying quantities of PBS (100 or 200 mL for rice hull or feed, respectively) were added to each bottle to create a 20% suspension. Bottles were shaken for approximately 10 sec, at which point they were allowed to settle overnight at 39.2°F. On the next day, supernatant was collected and multiple aliquots prepared for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at -4°F until qRT-PCR analysis was performed within 7 d of inoculation on one aliquot per sample bottle. The remaining three samples per bottle were stored at -112°F until further use. Dust samples were subsampled into 1 mL aliquots, and 4 mL of PBS was added resulting in a 20% suspension by volume. Samples were stored in a similar manner to feed and rice hull flush bottles, and supernatant pulled the following day to be analyzed via qRT-PCR. The remaining dust was stored in dry form at -112°F until initiation of the bioassay portion of the experiment.

Polymerase chain reaction (PCR) assays were performed at the Kansas State University Molecular Diagnostic Research and Development Laboratory. Briefly, fifty microliters ( $\mu$ L) of supernatant from each sample were loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburg, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions with one modification, reducing the final elution volume to 60  $\mu$ L. One negative extraction control consisting of all reagents except the sample was included in each extraction. The extracted RNA was frozen at -4°F until assayed by qRT-PCR. Reported values represent threshold cycle time (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until

viral genetic material was detected, thus representing lower quantities of genetic material in the original sample.

### *Infectivity Characteristics*

Bioassay samples were selected after qRT-PCR analysis, and included a composite positive and negative control sample that had been collected from both laboratory and production-scale mixers, untreated rice hull flush, 2% MCFA rice hull flush, Sal CURB rice hull flush, subsequent feed after the untreated rice hull flush, subsequent feed after Sal CURB rice hull flush, subsequent feed after the 2% MCFA rice hull flush, subsequent feed after the 10% MCFA rice hull flush, 10% MCFA rice hull flush collected from the discharge spout of the production-scale system, and subsequent feed after the 10% MCFA rice hull collected from the discharge spout of the production-scale system. Additionally, dust samples included in the bioassay were those collected from mixing surfaces after manufacture of  $10^4$  TCID<sub>50</sub>/g inoculated swine feed, dust collected after the 10% MCFA rice hull flush, and subsequent feed after the 10% MCFA rice hull flush. Supernatant samples were allowed to thaw prior to bioassay inoculation at room temperature, beginning approximately 3 h prior to inoculation. Dust samples were prepared by combining the three positive control dust samples into a single, homogeneous positive control dust sample. A total of three, homogeneous, dust samples (positive, 10% MCFA rice hull flush, and subsequent feed dust) were then each split into three 5.2 g aliquots, and then adding 20.8 g PBS to create a 1:5 suspension of dust to total mass, with a volume of approximately 25 mL each. A 1 mL sample of the suspension was sampled for qRT-PCR analysis, and the remaining solution was inoculated into the appropriate pig ( $n = 3$  pigs per dust type).

The experimental protocol for the pig bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee. Forty-two crossbred, 10 d-old pigs of mixed gender were sourced from a single commercial, crossbred farrow-to-wean herd with no known prior exposure to PEDV. Pigs received a dose of cefitiofur (Excede, Zoetis, Florham Park, NJ) just prior to transport to the research facility. Upon arrival, piglets were ear tagged, weighed and randomly assigned to bioassay treatment rooms. Fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV) using qRT-PCR analysis. To further confirm PEDV negative status, serum was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and TGEV antibody by ELISA conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before inoculation.

Pigs from each treatment were housed in separate rooms with independent ventilation systems. Rooms had solid flooring that was minimally rinsed to reduce risk of PEDV aerosols. Pigs were fed liquid milk replacer once daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each of 33 pigs (11 rooms) receiving supernatant samples were administered 20 mL of the PBS supernatant by orogastric gavage using a 10-gauge French catheter 0 days post-bioassay inoculation (dpi). Each of 9 pigs (3 rooms) which were inoculated with dust suspensions by administering the separated supernatant via orogastric gavage (approximately 5 to 10 mL), with the remaining solid fraction of the inoculum directly placed in the mouth of each pig at which point

they were stimulated to swallow. Rectal swabs were collected daily throughout the bioassay experiment from all piglets, and tested for PEDV RNA via qRT-PCR on d -2, 0, 2, 4, 6, and 7 dpi. Fresh small intestine, cecum, and colon were collected at necropsy at 7 dpi, along with an aliquot of cecal content. One section of formalin-fixed proximal, middle, and distal jejunum and ileum were collected for histopathology. Cecal content was evaluated for presence of PEDV genetic material via qRT-PCR. Tissue was processed and fixed in neutral buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin stain. One section of proximal, middle, and distal jejunum; and three serial sections from the piece of ileum (for a total of six sections of intestine) were evaluated by a veterinary pathologist blind to the treatments.

### *Statistical Analysis*

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

## **Results and Discussion**

### *Viral RNA Quantification*

After qRT-PCR analysis, the composite negative feed sample did not have detectable RNA, and composite positive control feed sample contained detectable PEDV genetic material (Table 2). Following a PEDV positive batch of feed in laboratory-scale mixers, 50% of the untreated rice hull flush samples had detectable PEDV RNA. The untreated rice hull flush reduced ( $P < 0.05$ ) the quantity of detectable RNA compared to the PEDV positive batch of feed. One Sal CURB treated rice hull flush sample was positive for PEDV genetic material, and 33% of the 2% MCFA rice hull samples had detectable PEDV RNA. Additionally, none of the 10% MCFA rice hull flush samples had detectable PEDV RNA. Chemically-treated rice hull flushes using Sal CURB and 10% MCFA reduced ( $P < 0.05$ ) the quantity of detectable RNA present in the rice hull flush samples compared to the untreated rice hull flush. However, the 2% MCFA rice hull flush did not reduce ( $P = 0.215$ ) the quantity of genetic material compared to the untreated rice hull flush. Importantly, no feed samples after an untreated or chemically-treated rice hull flush had detectable PEDV RNA.

After manufacturing a PEDV-positive batch of feed in the production-scale mixer and bucket elevator, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectable RNA. However, none of the rice hull flush samples collected from the mixer or subsequent feed samples from the mixer or bucket elevator discharge spout had detectable PEDV RNA. The presence of detectable viral RNA in the 10% MCFA-treated rice hull flush sample collected from the bucket elevator discharge spout provides evidence that bucket elevators are a significant source of cross contamination within feed manufacturing systems.

All pigs were free of PEDV genetic material in fecal swabs and PEDV-specific antibodies prior to initiation of the bioassay experiment. On 2 dpi, fecal shedding of PEDV

RNA was detected in positive control pigs. No other flush feed bioassay pigs had detectable RNA in fecal swabs throughout the study or cecal content collected at necropsy.

Dust collected after mixing the positive feed had a large quantity of viral RNA (Table 3). Following the inoculated batch of feed, dust collected immediately following the 10% MCFA rice hull flush batch had a reduced quantity of viral RNA, and subsequent feed following the 10% MCFA rice hull flush did not have detectable RNA. Pigs inoculated with the positive dust collected following mixing of inoculated feed were shedding PEDV by d 2 after oral inoculation and continued to shed through necropsy at 7 dpi. However, pigs inoculated with the dust from the 10% MCFA rice hull flush batches or the subsequent feed batch had no indications of PEDV infection.

Overall, the rice hull flush effectively reduced the amount of detectable RNA present compared to feed inoculated with PEDV, as expected. Chemical treatment of rice hulls with Sal CURB and 10% MCFA provided additional reduction in detectable RNA present in the rice hull flush samples. Feed manufactured following rice hull flushes did not contain PEDV RNA, therefore utilizing rice hull flushes would be a cost effective strategy to mitigate the risk of PEDV transmission via the feed manufacturing process. Finally, dust collected following the manufacture of PEDV inoculated feed contained a large quantity of PEDV RNA and was infective. Therefore, a high level of caution should be taken to limit and control dust created during feed manufacturing as it may serve as a vector in PEDV transmission.

**Table 1. Diet composition (as-fed basis)**

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

<sup>1</sup> HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

**Table 2. Effect of chemically-treated rice hull flushes on PEDV RNA detection and infectivity of samples collected in feed manufacturing equipment<sup>1</sup>**

Item	Rice hull treatment			
	Untreated	Sal CURB <sup>2</sup>	2% MCFA <sup>3</sup>	10% MCFA
Prevalence, % positive				
Negative feed	0.0 (0/3)			
Positive feed	100.0 (3/3)			
Laboratory-scale mixer				
Rice hull flush	50.0 (3/6)	16.7 (1/6)	33.3 (2/6)	0.0 (0/6)
Subsequent feed	0.0 (0/6)	0.0 (0/6)	0.0 (0/6)	0.0 (0/6)
Production-scale mixer				
Rice hull flush	---	---	---	0.0 (0/3)
Subsequent feed	---	---	---	0.0 (0/3)
Production-scale bucket elevator				
Rice hull flush	---	---	---	33.3 (1/3)
Subsequent feed	---	---	---	0.0 (0/3)
Cycle threshold, Ct				
Negative feed	45.0 <sup>a</sup> (-) <sup>4</sup>			
Positive feed	30.2 <sup>d</sup> (+)			
Laboratory-scale mixer				
Rice hull flush	41.4 <sup>c</sup> (-)	43.9 <sup>ab</sup> (-)	42.4 <sup>bc</sup> (-)	45.0 <sup>a</sup>
Subsequent feed	45.0 <sup>a</sup> (-)	45.0 <sup>a</sup> (-)	45.0 <sup>a</sup> (-)	45.0 <sup>a</sup> (-)
Production-scale mixer				
Rice hull flush	---	---	---	45.0 <sup>a</sup>
Subsequent feed	---	---	---	45.0 <sup>a</sup>
Production-scale bucket elevator				
Rice hull flush	---	---	---	42.0 <sup>bc</sup> (-)
Subsequent feed	---	---	---	45.0 <sup>a</sup> (-)

<sup>1</sup> Swine feed was inoculated with porcine epidemic diarrhea virus (PEDV) at a concentration of 10<sup>4</sup>TCID<sub>50</sub>/g and passed through laboratory-scale paddle mixers, followed by a rice hull flush, and subsequent batch of PEDV negative swine diet. Batch size was 5.5 lb with a mix time of 5 min.

<sup>2</sup> Sal CURB (Kemin Industries, Inc., Des Moines, IA) was added at recommended level of 6.5 lb/T.

<sup>3</sup> Medium chain fatty acid blend (1:1:1 ratio of caproic, caprylic, and capric acid) added on a wt:wt basis to ground rice hulls.

<sup>4</sup> (+) indicates 3/3 pigs were shedding PEDV genetic material at 2 dpi and continued to shed through 7 dpi and cecal content collected at necropsy contained PEDV genetic material while (-) indicates 0/3 pigs had detectable PEDV genetic material in fecal swabs or cecal content and remained negative throughout the full 7 d bioassay.

<sup>abc</sup> Cycle threshold means lacking common superscript differ (*P* < 0.05). Pooled SEM=0.85.

**Table 3. Effect PEDV RNA detection and infectivity in environmental dust samples<sup>1</sup>**

Item	Cycle threshold, Ct <sup>2</sup>	Infectivity <sup>3</sup>
Positive feed dust	29.4	+
10% MCFA rice hull dust	33.7	-
Subsequent feed dust	45.0	-

<sup>1</sup>Dust samples were collected from the laboratory and production mixers from non-feed contact surfaces.

<sup>2</sup>Positive feed dust, average of  $n = 3$ , 10% MCFA rice hull dust,  $n = 1$ ; subsequent feed dust,  $n = 1$ .

<sup>3</sup>Evaluated in a 10 d old pig bioassay with 3 pigs per dust type. (+) indicates 3/3 pigs were shedding PEDV genetic material at 2 dpi and continued to shed through 7 dpi and cecal content collected at necropsy contained PEDV genetic material while (-) indicates 0/3 pigs had detectable PEDV genetic material in fecal swabs or cecal content and remained negative throughout the full 7 d bioassay.

## Gilt Training for Electronic Sow Feeding Systems in Gestation<sup>1</sup>

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### Summary

An electronic sow feeding (ESF) system provides the capability of feeding group-housed gestating gilts and sows on an individual basis. One of the most critical and yet often neglected steps in making an ESF system a success is proper gilt training. Different farms have protocols adapted to their particular situation, but the overall goal of gilt training is to ensure that a high percentage of gilts learn how to utilize the ESF station before they are moved to gestation. There are three critical steps in proper gilt training. These include: 1) pre-training; 2) training once the gilts have been moved to the training pen with the ESF; and 3) a post-training period. To have a successful gilt training requires dedicated people who are patient, observant, and also who are able to establish a connection with the females. This frequently necessitates that one or two people are directly responsible for gilt training in an ESF system. This paper will illustrate some of the key gilt training steps involved with an ESF system.

Key words: electronic sow feeding, gilt training, group-housed gestating sows

### Introduction

The U.S. swine industry is facing a significant change in production practices regarding gestation sow housing. Many pork producers across the United States are shifting from housing gestating sows in individual stalls to a group housing system. This is driven by animal welfare concerns, state legislatures, and food companies' demands.

Electronic sow feeding (ESF) is one option for feeding gestating sows within a group housing system. Electronic sow feeding systems are a means to manage and monitor individual feed intake and provide opportunities to adjust feeding program strategies to better satisfy gestation nutrient requirements. However, in order to get the maximum benefits from the ESF systems, training of replacement gilts is a priority.

Gilts need to be comfortable with the entrance and exit gates of ESF feeding stations. If they are not comfortable and do not eat they may lose body condition and have poor

<sup>1</sup> Appreciation is expressed to the Thomas Livestock Company, Inc., Broken Bow, NE, for their help and expertise, and Tim Kurbis, New Standard, Inc, for help with the NEDAP system.

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reproductive performance. This may result in an increased gilt culling rate. Therefore, the following information will demonstrate key concepts of a gilt ESF training protocol.

## Procedures

The implementation of ESF systems requires a significant change in how the gilts are managed. Besides the reproductive management, the employees will have to control the feed system and train gilts to use the feeding stations before mating. Designated gilt training personnel must have a unique personality. The trainers must be able to observe how gilts behave and establish a connection with the animals. The trainers must be patient enough to let the gilts explore the environment and become curious about the station gates instead of pushing them forcibly through the system. Training needs to be a positive experience for the gilts. Many producers often underestimate the time it takes to properly train gilts.

The training is divided into three different stages, which include: pre-training, training, and post-training (Figure 1).

### *Pre-Training Stage*

Replacement gilts can be acclimated to ESF stations during the growing phase by using training gates. The main goal of the pre-training stage is to expose gilts to gates and allow them to become familiar with walking through gates. Thus, gilts will be expected to have less fear when facing the entrance gates of the feeding stations.

The acclimation to the ESF units can be performed in different ways. After entering the farm at approximately 11 wk of age, gilts are allocated into grower pens containing training gates. These methods can range from low input one-way gating to higher input methods that closely mimic the equipment and flow of gilts through the ESF system.

### Low Input

In this method, the grower pen is subdivided into areas that contain feeding areas to provide ad libitum access to feed. Between these subdivisions there are simple one-way gates (Figure 2). If gilts want to move between these areas, they have to pass through those gates.

The one-way gates utilized in this method may have bars suspended vertically to attach so as to allow free movement, to accustom gilts to cross a physical barrier (Figure 2). The major advantage of this method is that it requires a low input of equipment and labor. However, it is not necessary to flow between the different sub areas of the pen to get access to feed and water. Therefore, some gilts may never walk through the gates during the pre-training stage.

### Higher Input

In this method, the grower pen is subdivided by portable pen gates into 3 different areas: pre-feeding, feeding, and post-feeding area (Figure 3). Gilts stay in the pre-feeding area with gated access to conventional feeders.

Gilts will need to walk through these gates to move from the pre-feeding area to the feeding area, in order to get access to feed. After eating, gilts have to pass through training gates again to move to the post-feeding area, without being able to return to the other areas. In the first day at the grower pen, all gilts are kept in the pre-feeding area. Some gilts will pass through the gates at their own will. At the beginning of the second day, the farm staff will be able to identify the gilts that have not flowed to the feeding area. Thus the farm staff have to manually assist gilts that have not eaten into the feeding area. The process of ensuring all gilts flow through the gates every day is called “single pass.” In our experience this is performed for the first 7 d after gilts enter the area. Hereafter, the portable pen gate is removed and the pre- and post-feeding areas become a single common area.

For entry gates into the feeding area, similar gates as used in ESF stations are utilized. One type of gate requires gilts to physically open them (Figure 4). Initially, the left entrance gate remains opened at a 45° angle for the first wk in order to facilitate and encourage gilts to walk through them. As the gilts become accustomed to these gates, they can be completely closed.

Another type of gate used is set up with an electronic sensor that causes the gate to open automatically when a gilt gets close to it (Figure 5). Thereby, this gate facilitates and encourages gilts to move between those areas. These types of gates are easier to move than the ESF unit entry gates, since they do not require gilts to physically open them.

This method requires gilts to flow through a physical barrier to get feed access, making them learn by positive reinforcement. However, this method requires the farm staff to dedicate time to initially manually assist gilts into the feeding area, which increases labor demands. The major advantage is that with the single pass system, gilts can be easily monitored to determine the number of gilts acclimated to the system. Also, it is expected that a higher level of acclimatization to the ESF station will occur compared to the low input method since the single pass system mimics the flow through the ESF system.

The pre-training period in the grower pen helps to properly introduce gilts to the new feeding system. One-way gates are a good tool to help gilts grow accustomed to them.

### *Training Stage*

The primary purpose of this stage is to train gilts to eat at the ESF stations. Depending on the level of acclimatization in the pre-training stage, gilts may have to be trained to enter the ESF feeding stations as well. During this stage the gilts have undergone the final gilts selection process and an radio frequency identification (RFID) ear tag has been placed. A large number of gilts can be trained at the same time but adequate staffing must be provided during the training period. Also, in our experience it is recommended a period of 2 wk to be allocated for the gilt training process. A significant amount of the time commitment occurs in the first wk with additional time needed in the second week monitoring to ensure gilts are regularly using the feeding stations.

The gilt training pen design varies between swine production systems. The gilt training pen illustrated in this paper has a capacity to house 250 gilts, providing 19 sq ft/gilt.

The pen is equipped with 24 nipple waterers to provide ad libitum access to water and 6 standard ESF stations set up as learning stations. Each feeding station is designed for approximately 42 gilts. It is suggested dispensing approximately 50% water with every feed drop, increasing the rate of eating. The staff has to ensure that the water being provided in the equipment is working correctly.

A single pass system such as described for the pre-training phase that does not allow gilt movement directly from the exit of the feeding station back to the entrance area is critical. There should be a post feeding area for gilts to rest after eating, with gating to subdivide the pen into pre- and post-feeding areas. Twenty-four hours prior to training, gilts are moved to the gilt training pen. Once the group is there, the portable pen gate is shut to start a single pass through the feeding station. Depending on pen configuration, additional penning may be provided, limiting the gilts near the entrance of the feeding area in order to further encourage them to begin passing through the ESF system.

Some of the untrained gilts will enter the stations at their own will. With the single pass system, the next day, the trainer will easily know which gilts have not eaten. Our experience in a commercial sow herd using this system, along with the higher input pre-training system, indicates that the first day after placement in the pen, approximately 60% of the gilts need to be manually delivered to the feeding station. This percentage decreases with time and by the fifth day only 30% of gilts require manual assistance through the gates, and few require assistance after the eighth day.

*Days 1 to 4:* In addition to the pen gates that keep gilts near the entry portion of the feeding stations, the ESF left entrance gate is tied open for the first 4 days (Figure 6). The aim of it is to arouse the gilts curiosity regarding the stations, and consequently motivate them to walk through the ESF gates.

Also, during this time, the trainer has to calmly start guiding the gilts from the pre-feeding area into the feeding stations. A sorting board can be used to encourage them to the entrance gate if necessary. However, it is critical this is done in a calm and positive manner so that the gilt does not develop negative associations with the feeding station.

Once a gilt enters the feeding station, there should be a mechanism in place to close the exit of the station during the training. The purpose of it is to prevent gilts from running directly from the entry to the exit of the ESF unit without being trained. One example of a simple mechanism is to shut the exit of the feeding station with a sorting board (Figure 7).

Once the gilts are inside the ESF station, the entrance gate is locked. The trainer has to manually drop feed in the bowl to attract the gilts to the feed. In the ESF system exemplified in this paper, the maximum time between the gilts entering the station, and getting close to the bowl where their RFID ear tag is identified, is approximately 30 sec. If the RFID has not been read by the ESF station within 30 sec, the entrance gate opens. Therefore, the trainer needs to close the gate manually, and use a board to narrow the space between the entrance and the feed bowl (Figure 8).

During the training time, each manually encouraged gilt is allowed to stay in the station for a period of 5 min. After that, the mechanism used to close the exit of the station has to be removed to allow the gilts to exit the station. In this example, the trainer takes off the front board enabling the gilt to exit the feeding station. The majority of gilts will have eaten at least a small amount of feed after 5 min. After this 5 min period, gilts that did not get even close to the bowl are allowed an extra 5 min to begin consuming their feed allotment. In this case, the trainer has to calmly direct these gilts to the feed bowl by using his hand to gently scratch their back, encouraging forward movement (Figure 9).

Some of the gilts will get somewhat stressed with the sound of the feed dropping. The trainer has to leave them in the station for an extra 5 to 10 min, depending on the gilt, in order to make them feel comfortable and lose the fear of that environment.

Even after 10 min, some gilts may not eat. This period serves for the animals to explore and familiarize themselves with the environment without stress, independently of eating or not. Therefore, the gilt is released after 10 min even if no feed was consumed. Feeding strategies may vary between farms. However, a typical setting is for a feed allowance for the first 4 d during the training process at 4.4 lb/d per gilt and with a feed delivery speed to drop 0.13 lb/min. Therefore, a gilt that starts eating as soon as she enters the station would eat approximately 0.65 lb during the 5 min training period.

During the first wk, it is advised that gilts should be eating at least 20% of the feed allowance, or between 0.9 and 1.3 lb/d. On d 3, the trainer should obtain from the software a report of feed delivery per gilt. The same procedures of d 1 and 2 are repeated, but now the goal is to ensure that all gilts eat. The gilts that haven't gone through the ESF station on their own are gently persuaded towards the stations. The trainer makes sure a certain amount of feed is dispensed in the bowl to stimulate the gilts to eat.

However, during the first wk and specially the first day, gilts are not going to eat much feed. In our experience, the first day in the gilt training pen approximately 18% of the gilts haven't eaten, 40% of them ate until 1.1 lb, 30 % ate until 2.2 lb, and only 12% of them ate between 2.2 and 4.4 lb of the allotted feed. At the end of the first week, approximately 50% of the gilts were eating more than 4.4 lb and less than 10% remained eating 1.1 lb or less.

In the beginning of the process, training gilts might take as long as 6 h considering that approximately 150 out of 250 gilts may need manual assistance to enter the feeding stations. The pen gate that divides the pen in pre and post feeding areas can be opened after the training. Thus gilts can freely circulate in the pen and even walk through the ESF stations again. During this period, gilts can enter the ESF station again and stay there enough time to eat as much as they want.

The feed system is programmed to start a new cycle every day at a specific time. The staff has to close the pen gate and move all the gilts to the pre feeding area before the feed system resets, to monitor and identify the non-eaters the next day. It is suggested that the feed system be set to start another cycle in the late afternoon. This will provide enough time for the gilt training personnel perform the training and the single pass in a

timely manner, and allow gilts that have not eaten their full feed allowance to consume further amounts of feed on subsequent passes through the system. Also, around d 4 of training the trainer has to gradually close the feeding station gate. A spacer can be used to prop up the left entrance gate of the feeding station open at a 45° angle.

Additionally, if the majority of the gilts are waiting in front of the bowl for more feed and the trainer visually observes little left-over feed, the feeding strategy may be changed. One example is to set the feed strategy to 6.6 lb/d and the feed speed to 0.22 lb/min when the next feed cycle starts.

*Days 5, 6, and 7:* The same procedures are continued to ensure that all gilts learn to consume feed at the feeding stations. The time each gilt is allowed to stay in the station during training goes up from 5 to 10 min because fewer animals need to be trained to walk through the system. After training, the loafing area gate is opened again, and some gilts will pass through the station at their own will. One hour later, the trainer might look for the gilts that ate less than 20% of their feed allotment, and assist them through the system before another feed cycle begins. These gilts should be allowed to stay in the station for enough time to eat as much as they want.

*Days 8 and later:* Based on the daily feed report, the trainer should look for gilts have not learned how to use the ESF station and may need to consider culling these animals. Criteria we have seen used includes gilts either not eating for more than 3 consecutive days at the end of the first wk of training or low total feed intake for the first wk of training. The trainer has to pay attention to the fact that sometimes when gilts are in heat, they might not eat for two days. Thus, it is critical that the trainer be able to identify gilts that are displaying normal estrus signs and those that have not been trained to eat.

In the second week of training gilts that did not eat at least 40% of their daily feed allowance are targeted for manual assistance through the ESF station again, and gilt eating behavior is closely monitored to ensure they are operating the feeding system correctly. Also, these gilts are closely examined for injuries or other reason why their feed intake may be compromised. Again, feeding behavior is closely monitored and feed allotments increased. If most gilts are eating the allotted amount of feed, the feed strategy is changed to 8.8 lb/d, with the feed delivery speed increased. It is critical though that feed allotment is not increased too fast or there will be wasted feed and feed build-up in the feeding station.

*Days 9 and 10:* The trainer continues assisting gilts with a feed consumption lower than 40% through the system. Starting on d 10, the entrance gate of the feeding station is left completely closed. The individual training comes to an end, and the gilts have to pass through the system at their will. However, the trainer has to look at the daily feed report to detect early signs of gilts that are not eating. Typically, after 2 weeks of training, gilts are moved to an ESF pen or breeding stall for observation of signs of estrus.

### ***Post-Training Stage***

Again as during the training stage on the first day in this new environment, the staff should ensure that non-eaters are flowing through the feeding stations. After four days,

if there are any gilts that have a feed consumption less than a target of, for example, 40% of the feed allowance or 3.5 lb/day, they may be candidates for further subsequent training or culling.

### Conclusion

Gilt training to utilize the ESF system is a challenge for many pork producers using ESF. Often the time commitment required for training gilts in ESF systems is underestimated. Additionally, it is important to acclimate gilts to the feeding system during the growing phase. Individual producers may need to make adjustments according to their situation and facilities. However, it is critical that the training occurs in three stages, which include pre-training, training, and post-training. The use of gates in the pre-training stage, the single pass practice, and the individual feed intake tracking are important events that have to be performed. Gilt training programs are critical to successfully implement an ESF system. Training programs require a lot of patience and properly trained employees for success.

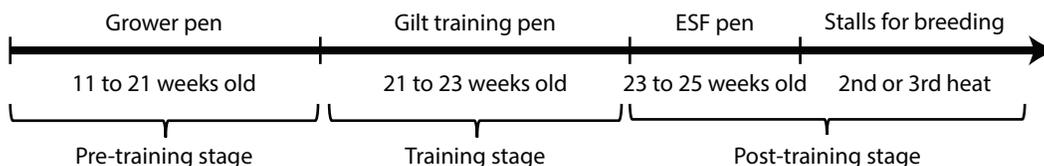


Figure 1. Diagram of the three stages involved with gilt training in ESF system.



Figure 2. One-way gate with bars suspended vertically.



Figure 3. Pre-feeding, feeding, and post-feeding areas.



Figure 4. Gate similar to ESF. Left entrance gate open at 45° angle.



Figure 5. Gate with electronic sensor to the feeding area.



Figure 6. Left entrance gate wide open.



**Figure 7. Front board to shut the exit.**



**Figure 8. Board to narrow the space to bowl.**



**Figure 9. Scratching a gilt to encourage forward movement.**

## Lessons Learned from Managing Electronic Sow Feeders and Sow Body Weight Data<sup>1,2,3</sup>

*L.L. Thomas, S.S. Dritz,<sup>4</sup> M.D. Tokach, R.D. Goodband, J.M. DeRouchey, and J.C. Woodworth*

### Summary

As the swine industry is transitioning from individual gestation stalls to different styles of group housing, new challenges are being presented for collecting data in the gestation barn. Electronic sow feeders (ESF) are computerized feeding stations that track and dispense feed for each sow that enters the feeding station. Individual intakes for sows can be recorded, which creates an opportunity for conducting nutrition studies in gestation. A research study was conducted on a commercial sow farm in central Nebraska, where sows were group-housed with ESF. A total of 74,114 feed intake observations and 663,204 sow weights were recorded during the study.

Feed intakes were downloaded daily, with unknown errors occurring during download 13 of 149 days. Intakes had to be downloaded prior to the system reset each day or the previous data would be deleted. Zeroes observed as feed intake values indicated the sow walked through the system, but did not consume any feed. Weights were automatically recorded and stored in system software for multiple weeks at a time. Numerous challenges were presented when attempting to determine accurate sow weights generated from this system, thus two weights were manually collected on all sows and used as reference weights. The reference weights were applied to the data set to eliminate inaccurate weights based on expected weight gains.

Using these data, we found that even with adequate training, parity 1 sows were reluctant to consume the assigned feed allowance immediately after placement into the pen as well as throughout the course of gestation. Parity 2 and 3+ sows had similar struggles immediately after placement. It is unknown what could be causing this type of behavior, however, as we continue to generate research within these types of feeding systems, we will continue to improve our knowledge of this system and ultimately improve animal performance.

<sup>1</sup> Appreciation is expressed to Thomas Livestock Company (Broken Bow, NE) for providing the animals and research facilities and to Tim Friedel, Steve Horton, and Jose Hernandez for technical assistance.

<sup>2</sup> Appreciation is expressed to New Standard US, Inc. (Sioux Falls, SD) for providing the scale system and to Tim Kurbis for technical assistance.

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Key words: sow, electronic sow feeders

## Introduction

As many production systems are transitioning from individual gestation stalls to different styles of group housing, there are new challenges for data collection in the gestation barn. Commonly referred to as electronic sow feeders (ESF), computerized feeding stations have been available for more than 30 years as a method used to control the variability of sow intake (Trottier and Johnston, 2001<sup>5</sup>), and now are becoming a powerful tool for sow research.

Electronic sow feeders typically have single enclosed feeding stations that can feed up to 60 group-housed sows per station. The stations are equipped with computers that track and dispense feed for each sow as she moves through the feeding station. The sows each have an ear tag that contains a radio frequency identification (RFID) transponder that identifies the specific sow to the system. This type of system is appealing to producers as it allows them to feed sows as individuals while in a group-housed setting. Electronic sow feeders are appealing to researchers because individual feed intakes can be recorded in some of these systems. This creates numerous research opportunities.

A research study was conducted on a commercial sow farm in central Nebraska, where sows were group-housed with ESF. Before leaving the feeding station and returning to the pen, sows walked over a scale where daily weights were collected. Following the completion of the study, 663,204 weight and 74,114 intake observations were recorded. Thus, data collection, and management were each crucial processes in making this study a success. Many lessons were learned along the way from managing ESF and sow body weight data. Therefore, the first objective of this review is to outline problems that emerged when collecting ESF and sow body data. The second objective is to better understand the intake patterns observed in grouped housed sows with ESF.

## Procedures

### *Group-Housing System*

Sows (Line 1050, PIC, Hendersonville, TN) were group-housed (approximately 260 sows per pen) from d 4 to 112 of gestation in dynamic groups (Figure 1). Each pen was equipped with 6 electronic feeding stations (NEDAP, The Netherlands) allowing for 45 sows per station. Pens also included a pen with a boar for automated heat detection and a holding area for sow separation. Heat detection is a separate station that automatically recognizes, marks, and separates sows based on heat. The sow separation system recognizes the right animals to be separated and automatically leads them to the holding area. In 3 selected pens, 1 scale (84" long × 20" wide; New Standard US Inc., Sioux Falls, South Dakota) was added to the alleyway following the individual feeding stations leading to the pen. The prototype scale would automatically collect sow weights through the attached antenna that would read the sow's RFID tag, similar to the ESF. Weights were recorded as the sow placed her hind legs on the platform.

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<sup>5</sup> Trottier, N. L., L. J. Johnston. 2001. "Feeding gilts during development and sows during gestation and lactation." Swine Nutrition. 2nd ed. AJ Lewis and LL Southern, ed. CRC Press, Boca Raton, FL (2001): 725-769.

### *Data Collection*

Daily feed intakes were manually extracted through NEDAP software. The software didn't have long-term storage and if feed intakes were not extracted daily those intakes would be deleted. The feeding system reset at 2:00 p.m. daily, therefore intakes had to be downloaded prior to this time. The closer intake was downloaded to system reset, the more intakes were recorded. There were only 13 of 149 d when intake data were not downloaded successfully due to unknown system errors. The system appeared to be downloading but when users tried to retrieve the file, no data were found. Attempts were made to retrieve the data again, however, downloads were performed at 1:00 p.m., and most data could not be retrieved before system reset. Feeding stations were calibrated once weekly following standard farm procedures.

After 5 days of downloading intake data, values of 0 were observed for intake. Our concern was how could a sow have an intake of 0 if she walks through the feeding station? We were assuming that when her RFID was read, feed would be dispensed. After further investigation, we learned that there was one antenna in the feeding station, located at the feeder. A sow may enter the feeding station if the gate is unlocked. The gate is unlocked when the station is not occupied or if the sow within the station has already received her daily allowance of feed. When the sow enters into the station and gets her head close enough to the feeder or in the feeder to be read by the antenna, feed will be dispensed. However, if the sow doesn't remain in this area and leaves the feeding station, feed will not be dispensed. Thus, values of zero for feed intake may be recorded.

Weights were automatically recorded and stored in the system software by day for multiple weeks at a time. A total of 8 of 149 d of weights were not extracted for specific pens due to human error (computer chips were dropped in the pit or not cleared properly). The scales were calibrated every week during the time of feed calibration.

Sows had to walk across the scale as they moved from the feeding station and back into the pen. There was great variability in the weights being generated on a single day for individual sows. This forced us to pay close attention to how sows moved across the scale and determine a method that could be used to eliminate inaccurate weights.

In many cases, more than one sow was on the scale at a time. The sow in front had two front legs off the scale while the sow behind her only had her two front legs on the scale. During times of activity (feed calibration, reset of feeding stations, workers in the pen, etc.) feeding stations were almost always occupied by sows that already received their daily allowance of feed. This increased traffic through the alleyway causing sows to move quickly across the scale. There was discussion of adding a panel to the front of the scale to possibly slow the sows down during these times, however; some feared this might cause them to move too slowly and cause an unhealthy environment for the sows.

As a sow moves across the scale, the antenna reads the RFID and continues to record weights until the next responder tag is read. Younger sows and gilts, who were not as long as older sows, would be standing on the back end of the scale but not far enough forward for the antenna to read the RFID. Thus, these weights were recorded on the previous sow. The antenna was adjusted forward in attempt to account for this, which improved readings.

The antenna on the scale was able to read through the panels of the scale and if a sow were lying on the opposite side of the scale, in the pen, her RFID would be read. However, once another sow was on the scale (in a position close enough to the antenna), her RFID was read and recorded properly. In addition, if a sow in the pen was lying against the panel adjacent to the scale, this impacted the accuracy of weights. The impact was greatest when multiple animals were nesting in this area. To prevent this and the previous concern from occurring, sternum bars can be added to the pen to prevent sows from lying or nesting in this area.

The scale system generated a score that indicated the number of consecutive stable measurements (1 point per measurement, 1 measurement per 250 ms). A high score was thought to generate a more credible weight; however, using this method to determine accuracy in weights was not successful because we had no way of knowing if all 4 feet were on the scale. The score may have been high indicating she was stable on the scale for a long period of time but she may have only been half way on the scale.

Each of these uncertainties contributed to the variability in daily weight collection. Therefore, manual weights were collected on all sows at the beginning and end of gestation. These weights were then used to eliminate inaccurate weights in the data set based on predicted gains.

### *Data Management*

During collection of these data, it became obvious that good data management would be imperative to properly analyze the large data set. Preparing data for analysis in addition to determining a method to accurately and confidently eliminate faulty sow weights from the data set was a major concern.

Through trial and error, it was determined advantageous to keep all data sets separate. Combining data within Excel is not recommended due to the size of these data sets and the risk of losing data. Handling each data set individually and then carefully merging them with statistical software proved to be most successful. It is important to note that there are cases when intakes and weights are missing for multiple days of gestation. In these cases, the intake data provide parity, and day of gestation. Thus if a sow did not eat, this information for that particular day of gestation would not be recorded. This presented challenges when attempting to model the data. Creating a spreadsheet that contained the sow identification number, parity, and date for d 4 to 112 of gestation eliminated this problem. This spreadsheet was then merged with the intake data file for all sows to have intake data for every day from d 4 to 112 of gestation.

Before merging the weights with the remaining data, it was necessary to eliminate incorrect weights from the data set. For this process, reference weights were collected and the following steps were applied to the data set to eliminate inaccurate weights.

- If the recorded weight was less than 220.5 lb (100 kg), the value was deleted as it was assumed to be inaccurate. The 100 kg minimum weight was selected as being well below the minimum weight for replacement gilts. Trottier and Johnston reported in 2001 that a target weight range of 245 to 265 lb at mating on second estrus was reasonable in gilts. PIC currently recommends the ideal live weight at first service to be 300 to 320 lb.

- Average daily gain (ADG) was calculated from the two reference weights for each sow.

$$\text{ADG} = (\text{Weight 2} - \text{Weight 1}) / (\text{Date 2} - \text{Date 1})$$

- Using ADG, a predicted weight was calculated based on the initial known weight and day of gestation.

$$\text{Predicted weight} = (\text{Weight 1} + (\text{ADG} * d)); \text{ where } (d) \text{ is calculated as the difference in days between the actual weight and the reference weight.}$$

- The ratio of predicted weight to the actual weight was determined and if the actual weight was 5% above or below the predicted weight, the weight was deleted (Figure 2).

$$\text{Ratio} = \text{Predicted weight} / \text{Actual weight}$$

Following these steps, the weight data set was reduced from 663,204 to 160,405 observations. The elimination of 502,799 observations reinforces the great variability in sow weights and how most of these weights were inaccurate.

In addition to these calculations, time adjustments were made to the weight data set. The ESF system resets at 2:00 p.m. daily and therefore the dates and times recorded for the weights did not correspond to the dates and times recorded for the intakes. Using the recorded times of data collection, data sets were adjusted to the same time of day, eliminating the problem.

A total of 135 sows were removed from the study. Of these sows, 40 were removed due to death or culling decisions by the farm. The remaining sows were removed based on missing intake values (greater than 3 consecutive days). Recall, errors occurred during download for feed intakes on 13 days. We assume the sows still consumed their daily feed allowance on each of these days and thus, replaced these missing values. After doing this, we removed sows who missed greater than 3 consecutive days of intake, indicating she has been removed from the pen. Sows were removed from the pen primarily for lameness or illness. In some cases, the sow returned to the pen later in gestation but we were unable to track her intake outside of the pen, thus these animals were removed from the study.

### *Data Analysis*

At the conclusion of this trial, files containing weights, intakes, progeny data, and backfat measurements were merged to create a master file containing 194,162 observations with 37 variables. With the help of statistical software, it becomes relatively simple to analyze any of the 37 variables. Some variables were common among the files (RFID) and were combined as they were merged; however, many were different, thus contributing to the large number of variables in the final data set (initial backfat, total born, etc.) We were not only able to analyze the data for the purpose of which this study was conducted, but also analyze the behavior of these animals in this production system.

Most producers would agree that when operating with an ESF, getting the animals acquainted with the system and on full feed can be a challenge. It is becoming common practice for producers with ESF to develop gilt training programs. Some programs are

more elaborate than others but the goal is to teach the gilt or sow how to use the system. Gilts are unfamiliar with the equipment and can be reluctant to enter or remain in the station and, thus, not eat. Reduced feed intake in gestation is a large concern for producers, thus maintaining the set feed intake is important.

In this system, gilts receive two weeks of extensive training prior to breeding and placement in gestation group housing. Prior to this training, gilts become familiar with the ESF system as they move through the nursery and the gilt development unit, through the use of pre-training practices (enclosed electronic scale and one-way gates). Gilts and sows were added to the pen 4 d post breeding and remained there until d 112 of gestation. Gilts and sows received 4.4 and 5.0 lb/d of feed while animals deemed as skinny, based on body condition scores evaluated by the farm employee, received 6.6 lb/d.

Data were grouped by parity: 1, 2, and 3+. We determined the average feed consumed based on the day of gestation, focusing specifically on intake within the first 10 days in the pen. We assumed that within this initial time period we will see the largest reduction in feed intake, attributed to the feeding system. The data show us that each parity group was consuming less feed than what was being offered as they entered into the ESF system, especially parity 1 and 2 sows (Figure 3). By day 7 of gestation, parity 2 and 3+ sows had recovered but parity 1 sows were still not consuming the allotted amount of feed. This is likely because older sows were more familiar with the system. During the first 10 days in the pen, sow mixing and subsequently fighting may have affected feed intake, but the impact is unknown.

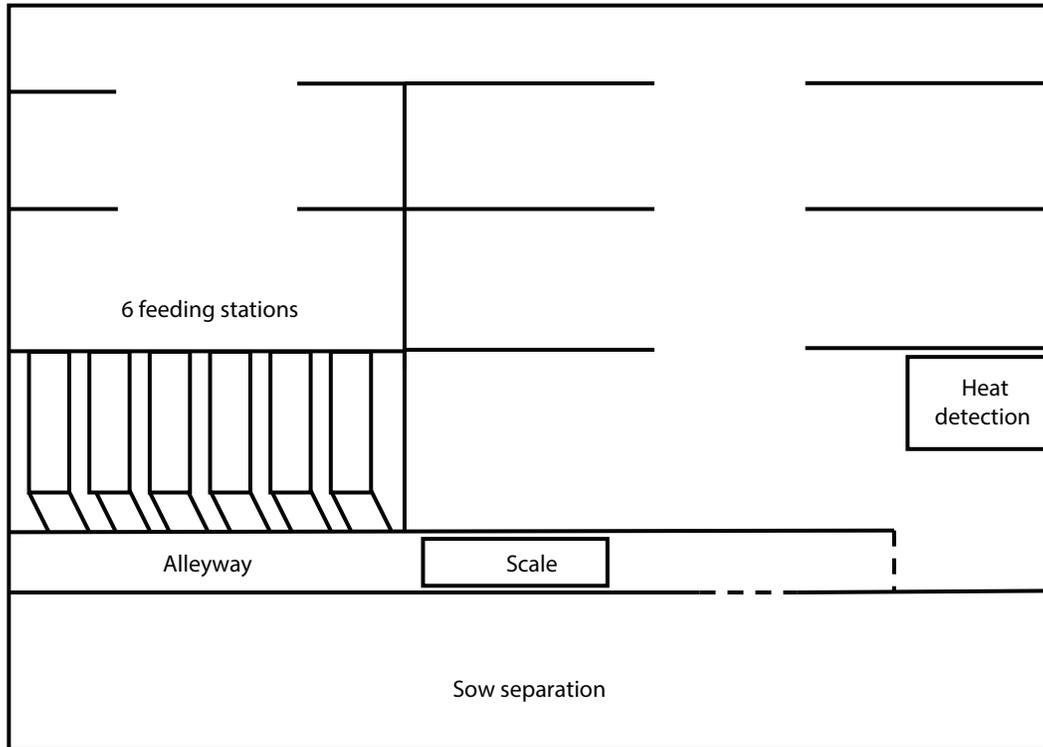
Scatter plots were created, grouped by parity group, to show the amount of feed consumed over the course of gestation. Each dot represents the intake of one sow for that particular day of gestation. Intake variability is high in parity 1 sows and within the first few days in the pen. We again see a majority of the animals eating less than what is offered (Figure 4). Also, many animals are off feed or eating less than their daily allowance throughout the course of gestation. It is important to note that although each sow represents one dot, many of these dots overlap, thus it may appear to be one single dot, but it represents numerous animals. When we move to parity 2 (Figure 5) and 3+ (Figure 6) sows, we see a large improvement in feed intake in comparison to parity 1 sows. The sows begin to consume their daily allowance much faster than the parity 1 sows. These animals also maintain a high feed intake throughout the course of gestation with less variability.

The same approach was used generate scatter plots to show the increase in weight observed through gestation for parity 1 (Figure 7), 2 (Figure 8) and 3+ sows (Figure 9). For parity 1 females, there appears to be a subset of gilts with greater weights in comparison to the others. These differences disappeared in parity 2 sows but appeared again for parity 3+.

## Results and Discussion

There are still many unknowns with this type of group housing feeding system. Our data indicate that even with adequate training, parity 1 sows were reluctant to consume the full amount of feed and remain at full feed for the course of gestation. Although fewer, there were numerous parity 2 and 3+ animals that appeared to have similar

struggles. Could this be influenced by the social structure in the pen? Are these animals not receiving enough training? Is behavior influenced by ESF design? As we continue to generate research from these types of feeding systems, we will continue to improve our knowledge of this system and ultimately improve animal performance.



**Figure 1. Group housing design where research study was conducted.**

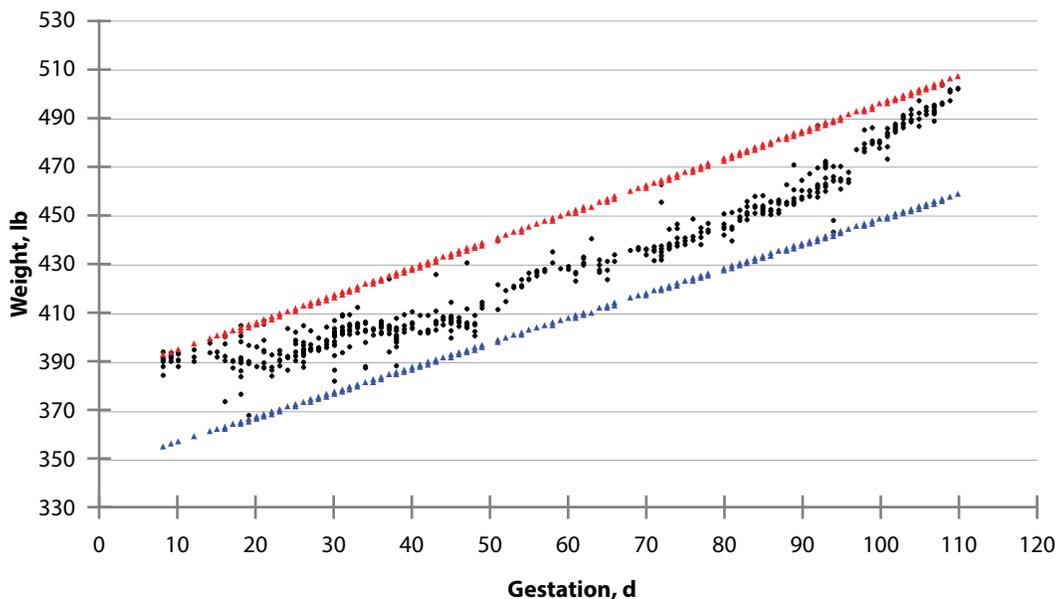


Figure 2. Individual sow BW throughout the course of gestation. The black dots (middle section) indicate weights obtained throughout the study. The red (top) and blue (bottom) lines were calculated based on the reference weights manually collected and used to determine ADG that could then be used to predict sow BW. Weights obtained 5% above (red/top line) or below (blue/bottom line) the ratio of predicted to actual weight, were deleted and deemed inaccurate.

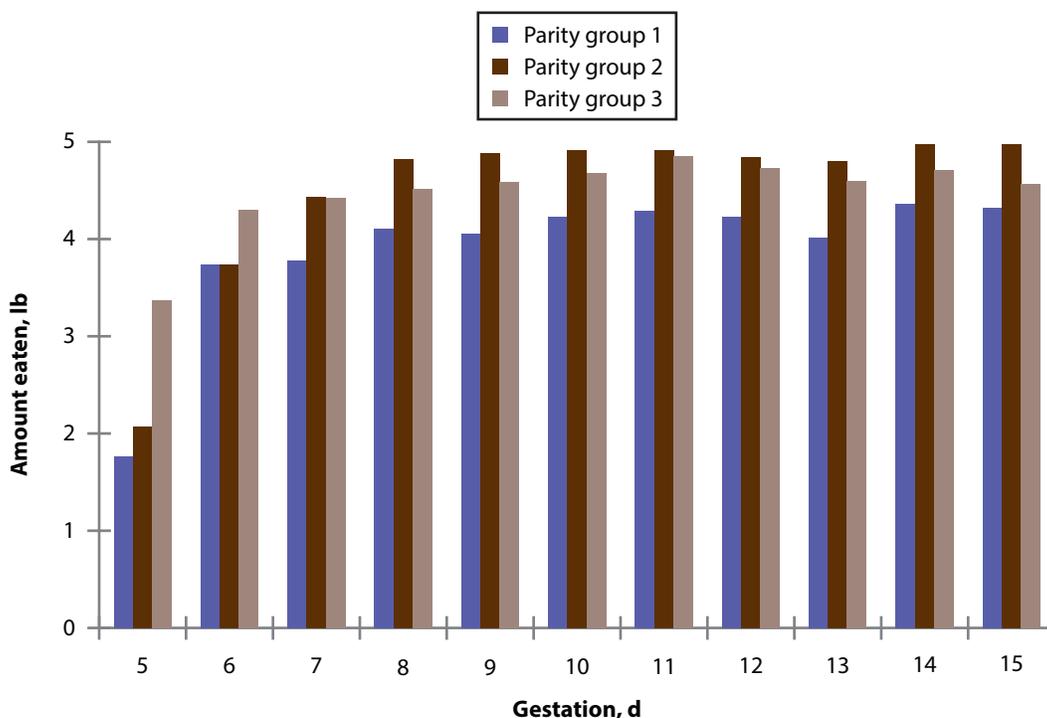


Figure 3. Average feed intake (lb) for all sows, grouped by parity group, for days 5 to 15 of gestation. Gilts and sows received 4.4 and 5.0 lb/day of feed while animals deemed as skinny, based on body condition scores evaluated by the farm employee, received 6.6 lb/d.

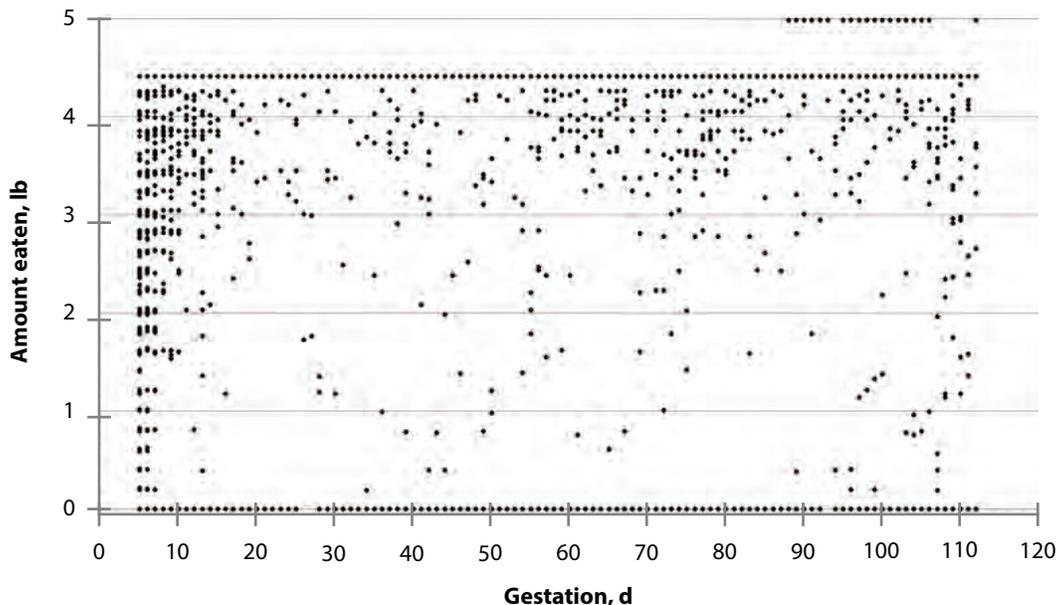


Figure 4. Daily feed intake (lb) from day 5 to 112 of gestation on all parity 1 sows. Each dot represents an individual sow but dots may overlap. All gilts received 4.4 lb/day of feed with the exception of 7 gilts that received 5.0 lb/day at day 112 of gestation and 1 gilt that received 5.0 lb/day from day 88 to 106 of gestation.

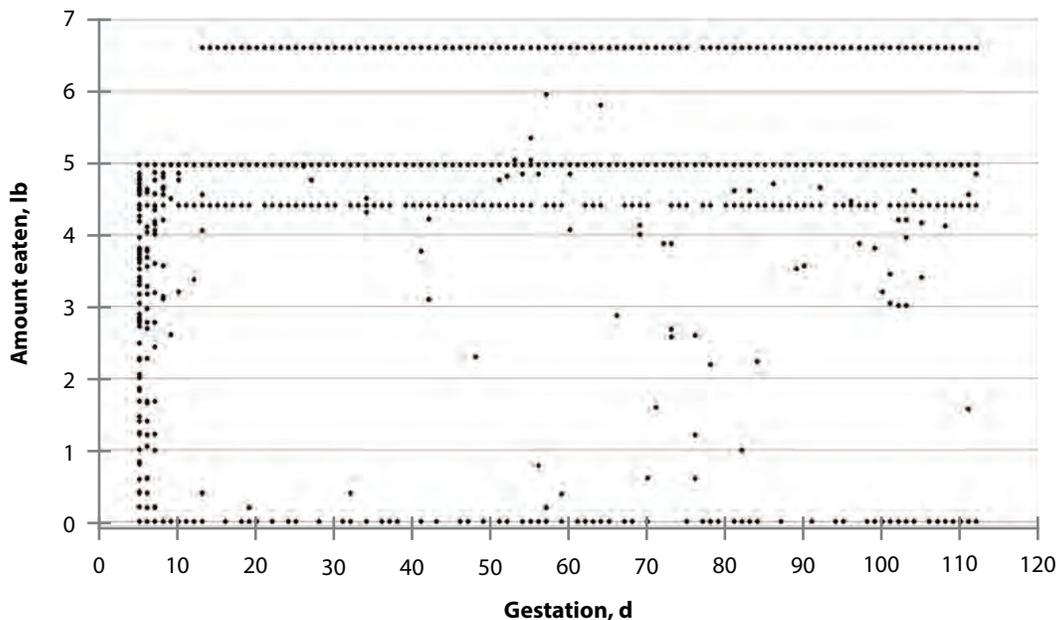


Figure 5. Daily feed intake (lb) from day 5 to 112 of gestation on all parity 2 sows. Each dot represents an individual sow but dots may overlap. Parity 2 sows of ideal body condition received 5.0 lb/day of feed and those deemed skinny (3 sows) received 6.6 lb/day of feed. One sow was set at 4.4 lb/day of feed.

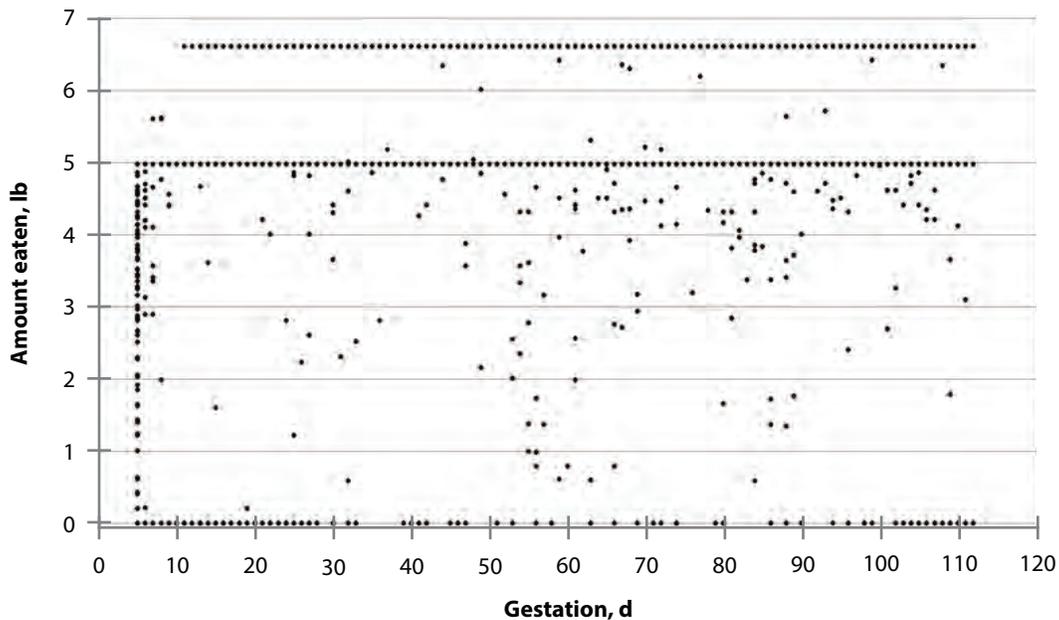


Figure 6. Daily feed intake (lb) from day 5 to 112 of gestation on all parity 3+ sows. Each dot represents an individual sow but dots may overlap. Parity 3+ sows received 5.0 lb/day of feed and those deemed skinny (9 sows) received 6.6 lb/day of feed.

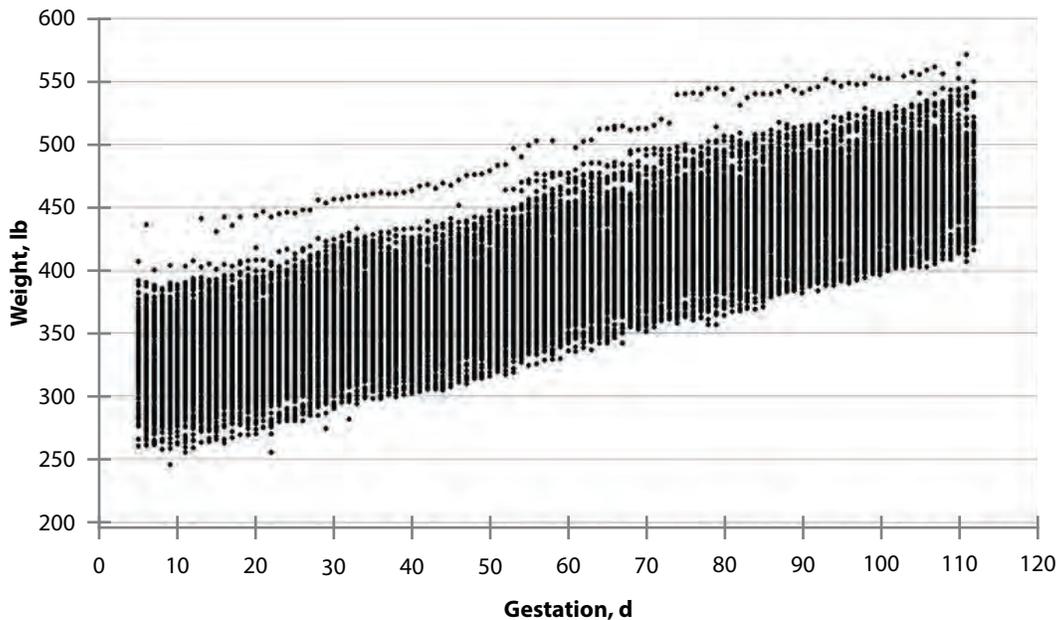


Figure 7. Daily BW (lb) from day 5 to 112 of gestation on all parity 1 sows. Each dot represents an individual sow but dots may overlap.

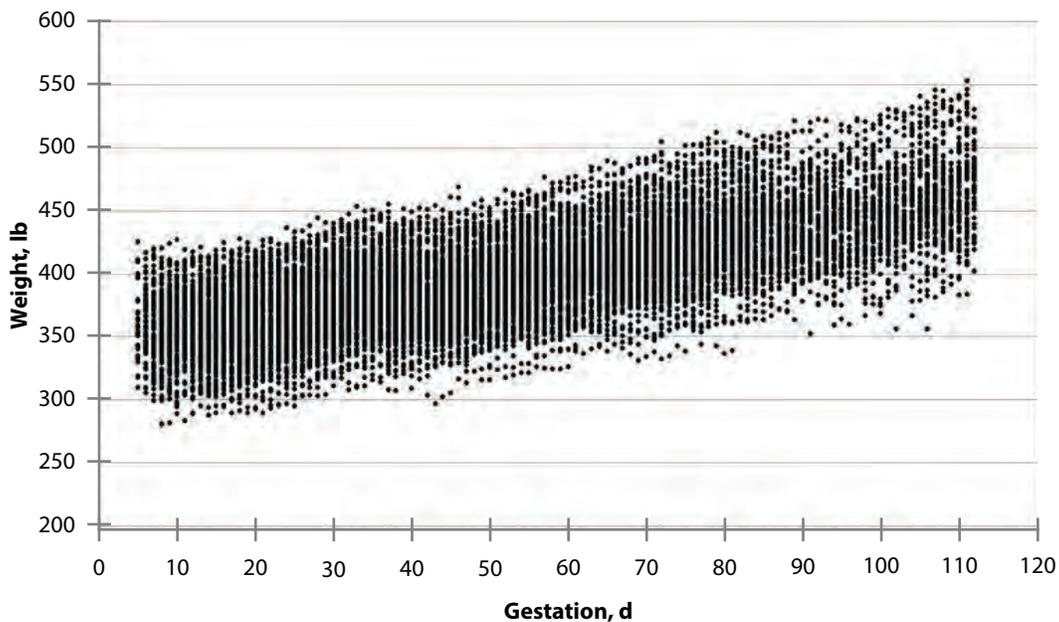


Figure 8. Daily BW (lb) from day 5 to 112 of gestation on all parity 2 sows. Each dot represents an individual sow but dots may overlap.

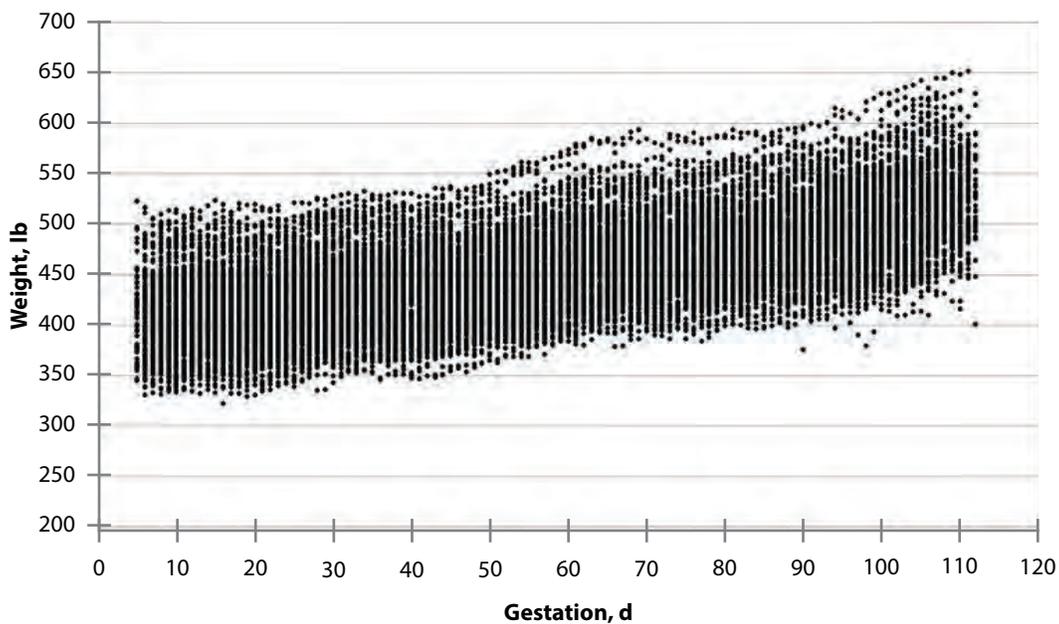


Figure 9. Daily BW (lb) from day 5 to 112 of gestation on all parity 3+ sows. Each dot represents an individual sow but dots may overlap.

## Generating an Equation to Predict Post-Farrow Maternal Weight in Multiple Parity Sows<sup>1,2</sup>

*L.L. Thomas, S.S. Dritz,<sup>3</sup> R.D. Goodband, M.D. Tokach, J.M. DeRouchey, and J.C. Woodworth*

### Summary

Post-farrow maternal weight is required when partitioning maternal and fetal weight gains throughout gestation. Equations were developed from the analysis of 150 females (Line 1050, PIC, Hendersonville, TN) to predict the weight of conceptus by difference of pre- and post-farrowing weight change in multi-parity sows. Females were individually weighed as they were moved into the farrowing house at d 110 to 112 of gestation and again at 12 to 24 h after farrowing. Data were divided into 4 groups: (1) parity 1 sows; (2) parity 2 sows; (3) parity 3 sows; and (4) parity 4+ sows. Each group tested 3 predictor variables: pre-farrow weight, total born, and difference in days between the pre- and post-farrow weights. Prediction equations were then developed using models with significant terms based on the Bayesian Information Criterion (BIC). The optimum equations to predict maternal body weight were similar for all parities except for the intercept (b) and can be described as:

$$\text{Post-farrow maternal body weight (lb)} = b + (0.897 \times \text{pre-farrow BW, lb}) - (1.118 \times \text{total born, n}) + (6.87 \times \text{days pre to post-farrow, d})$$

Where the intercept (b) for parities 1, 2, 3, and 4+ were -5.93, 5.15, 11.90, and 32.31, respectively.

The prediction equations were then used to estimate post-farrow maternal BW using 332 mixed parity sows (PIC 1050). Pre-farrow weights were taken on d 113 of gestation and maternal BW was taken within 24 h of farrowing. On average, the predicted post-farrow maternal BW was overestimated by 3.3 lb of the actual. Management practices differed in how females were fed from the validation experiment, possibly contributing to the overestimating of post-farrow maternal BW. This indicates that further evalua-

<sup>1</sup> Appreciation is expressed to Thomas Livestock Company (Broken Bow, NE) for providing the animals and research facilities. Additional appreciation to Tim Friedel, Steve Horton, Jose Hernandez, and Rebekah Spader for technical assistance.

<sup>2</sup> This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30336 from the U.S. Department of Agriculture National Institute of Food and Agriculture.

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tion of the equation is needed to see if the difference is due to litter size, parity distribution, or feeding management practices.

Key words: sows, post-farrow maternal weight

## Introduction

A successful gestation feeding program is one that yields a large, vigorous litter of pigs and a healthy sow equipped with adequate mammary development and body nutrient stores to produce large quantities of milk for the suckling litter. Variations in body size, productivity, and environmental conditions dictate different daily concentrations of nutrients to satisfy the sow's requirement. Models have been developed for sow nutrient requirements in gestation (NRC 1998,<sup>4</sup> NRC 2012<sup>5</sup>). These models attempt to partition nutrient requirements into three components: sow maintenance, products of conception, and maternal weight gain.

The process of reproduction from conception to weaning involves both homeostatic and homeorhetic control of nutrient partitioning (Dourmad et al., 1999<sup>6</sup>). The maintenance of the body is the main homeostatically controlled process, whereas the products of conception and maternal weight gain are regulated through homeorhetic controls. Maintenance of the sow and growth of the conceptus receive the highest priority for nutrients. When these two needs are satisfied, any remaining nutrients can then be deposited in maternal tissue (fat and protein deposition). If nutrient supplies are not sufficient, body proteins and lipids are mobilized to support maintenance requirements and conceptus growth.

Thus, optimal sow performance and longevity require a careful approach to determining the nutrient requirement during pregnancy in order to control the sow's body reserves. Overfeeding in gestation can cause increases in weight and body condition of the sow at the end of pregnancy, causing farrowing difficulties, decreased appetite in lactation, and increasing the risk of heat and environmental stress in the farrowing house (Dourmad et al., 1999). Underfeeding in gestation lowers body fat reserves at farrowing and at weaning, lowers conception rate, delays return to estrus, and ultimately decreases sow longevity.

Previous models provide equations for the prediction of sow maintenance requirements, products of conception, and maternal weight gain; however, when determining products of conception, a post-farrow maternal BW, in addition to pre-farrow BW, is required.

Unfortunately, in commercial research, removing a newly farrowed sow from a farrowing crate and walking her to a scale to be weighed, can be challenging. Producers express concerns when moving sows in and out of the farrowing crate after farrowing because of its impact on pre-wean mortality. Therefore, a prediction equation is necessary to estimate post-farrow BW from pre-farrow BW to determine products of conceptus.

<sup>4</sup> NRC. 1998. *Nutrient Requirements of Swine*, 10<sup>th</sup> Rev. Ed. Washington, DC: National Academy Press.

<sup>5</sup> NRC. 2012. *Nutrient Requirements of Swine*, 11<sup>th</sup> Rev. Ed. Washington, DC: National Academy Press.

<sup>6</sup> Dourmad, J.Y., L. Noblet, M.C. Pere, and M. Etienne. 1999. Mating, pregnancy and prenatal growth. Pp. 129-152 in *Quantitative Biology of the Pig*, I. Kyriazakis, ed. Wallingford, UK: CABI.

The objective of the present study was to develop a model that can predict post-farrow maternal BW in order to determine maternal and fetal weight gains throughout gestation.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at a commercial sow farm in central Nebraska in early spring, 2016. Females were individually housed from weaning until d 4 after breeding. They were then placed in pens with 260 sows per pen and 22 sq. ft per sow and 21 sq. ft per gilt until moved into the farrowing house. Each group pen was equipped with 6 electronic sow feeding stations (NEDAP, The Netherlands) and 28 nipple waterers to provide ad libitum access to water.

Between d 110 to 112 of gestation, 150 females (Line 1050, PIC, Hendersonville, TN; 46 gilts and 104 sows) were moved to the farrowing house and provided ad libitum access to feed and water. The average parity for sows after farrowing was  $3.0 \pm 1.9$  (mean  $\pm$  SD). The gestation and lactation diets were corn-soybean meal-based and presented in meal form. All nutrients met or exceeded the NRC (2012) recommendations. Females were weighed individually as they were moved from gestation into the farrowing rooms and again at 12 to 24 h after farrowing.

PROC MIXED in SAS (SAS Institute, Inc., Cary, NC) was used to develop prediction equations. The statistical significance for inclusion of terms in the model was determined at  $P < 0.05$ . Further evaluation of models with significant terms was then conducted based on the Bayesian Information Criterion (BIC). A model comparison with a reduction in BIC of more than 2 was considered improved (Kass and Raftery, 1995<sup>7</sup>). The fixed effects evaluated were pre-farrow BW, total born, difference in days between the pre- and post-farrow BW, and parity group (Parity 1, 2, 3, and 4+). The random effect evaluated was the date when the post-farrow BW was obtained. There was no total born by parity group interaction or quadratic response of total born, thus these terms were removed from the model. The final model contained pre-farrow body weight, total born, the difference in days between the pre- and post-farrow BW and parity as input variables.

## Prediction Equation Evaluation

To evaluate the prediction equation used to estimate post-farrow maternal body weight, a data set with a total of 332 mixed parity sows (PIC 1050) was used. Pre-farrow weights were obtained on d 113 of gestation and post-farrow maternal BW were taken within 24 h of farrowing. Sows were given ad libitum access to water but feed intake was limited to 6 lb/d. Agreement was measured using a paired t test to evaluate the difference between actual and predicted weights. Limits of agreement were calculated.

## Results and Discussion

The pre-farrow weights and post-farrow maternal BW ranged from 419 to 697 lb and 357 to 680 lb, respectively. Parity after farrowing ranged from 1 to 7 and total born

<sup>7</sup> Kass, R.E., and A.E. Raftery. 1995. Bayes Factors. *J. Am. Statist.* 90:773-795.

ranged from 7 to 22 piglets. The difference in days between the pre-and post-farrow weights ranged from 1 to 7 d (Table 1).

Significant single-variable models used to predict post-farrow maternal BW included pre-farrow BW, difference in days between the pre-and post-farrow BW, and parity group ( $P = 0.001$ ). Total born was not statistically significant in the model ( $P = 0.072$ ), but reduced BIC, indicating a better fit and was therefore included in the final model. When evaluating bias for all 4 parity groups, the final equations tended to overestimate the weight gain of the lighter sows and underestimate the weight of heavier sows, especially for younger parity groups (Figure 1).

The optimum equations to predict maternal body weight were similar for all parities except for the intercept (b) and can be described as:

$$\text{Post-farrow maternal body weight (lb)} = b + (0.897 \times \text{pre-farrow BW, lb}) - (1.118 \times \text{total born, n}) + (6.87 \times \text{days pre to post-farrow, d})$$

Where the intercept (b) for parities 1, 2, 3, and 4+ were -5.93, 5.15, 11.90, and 32.31, respectively.

Prediction equations are tools that can become an integral part of a pork enterprise; however, it is essential that they are used correctly to prevent the generation of faulty information. It is important to realize that the equations are valid only as long as the input variables consist of values within the ranges used to generate the predictive equation.

The sows from this farm were provided ad libitum feed once they were placed into the farrowing crate. Therefore, the days spent in the farrowing crate prior to farrowing becomes important in predicting post-farrow maternal BW. The model predicts that for every day in the farrowing crate prior to farrowing, the sow gains 6.9 lb of BW. The body weight gain during this time is attributed to the conceptus and sow maternal gain. The model also suggests that as parity increases, the sow loses less weight and starts to progressively gain weight. We expect that gilts in comparison to older sows would increase their maternal body size at a faster rate compared to older sows if they consumed the same amount of feed; however, our model tells us that parity 1 sows are losing more weight in comparison to parity 2+ sows. This could be because gilts under consume and sows over consume what is required for their respective maternal and conceptus needs.

The range of prediction equation input variables derived from the validation experiment and the actual and estimated post-farrow maternal BW are presented in Table 2. Pre-farrow body weight, total born, difference in days between the pre- and post-farrow BW, and parity were used as input variables in the model to predict post-farrow maternal BW and then compared to the actual post-farrow maternal BW. On average, the predicted post-farrow maternal BW was 3.3 lb greater than the actual with 95% confidence interval -5.35 to 17.8 lb (Figure 2). The statistical difference was significant ( $P = 0.002$ ) between the actual and the estimated post-farrow maternal BW. The limits of agreement (-41.6 and 35.1 lb) also lead us to believe that the predicted post-farrow maternal BW was overestimated compared to the actual. Although the statistical differ-

ence between the actual and estimated post-farrow maternal BW was significant, there is evidence to believe that there is no biological difference. When applying this difference (3.3 lb) to sow gestation models, the impact on daily maintenance requirement and expected maternal gain is a difference of 32 kcal and 0.01 kg, respectively.

It is important to note that the validation experiment was conducted at a campus research facility among 11 farrowing groups. Management practices differed in how the females were fed in the farrowing house, with sows from the validation experiment receiving up to 6 lb per day compared to the sows used to develop the prediction equation receiving ad libitum access to feed. Therefore, the difference between the predicted vs. actual post-farrow maternal BW may be attributed to these varying factors. This indicates that further evaluation of the equation is needed to see if the difference is due to genetic background or feeding management practices.

Equations incorporating appropriate criteria to estimate post-farrow maternal BW will allow us to partition differences in maternal weight gain throughout gestation as well as that of the conceptus. This will allow for a better understanding of where dietary energy intake is utilized and how much is deposited as maternal tissue.

**Table 1. Descriptive statistics for data included in the evaluation<sup>1</sup>**

Item	Mean	SD	Minimum	Maximum
Pre-farrow weight, lb	520.1	53.06	419	697
Maternal body weight, lb	490.5	61.35	357	680
Parity	3.0	1.87	1	7
Total born	16.1	2.77	7	22
Days pre- to post-farrow, d <sup>2</sup>	4.2	1.37	1	7

<sup>1</sup>A total of 150 females (PIC 1050) were used to develop a prediction equation to estimate post-farrow maternal weight.

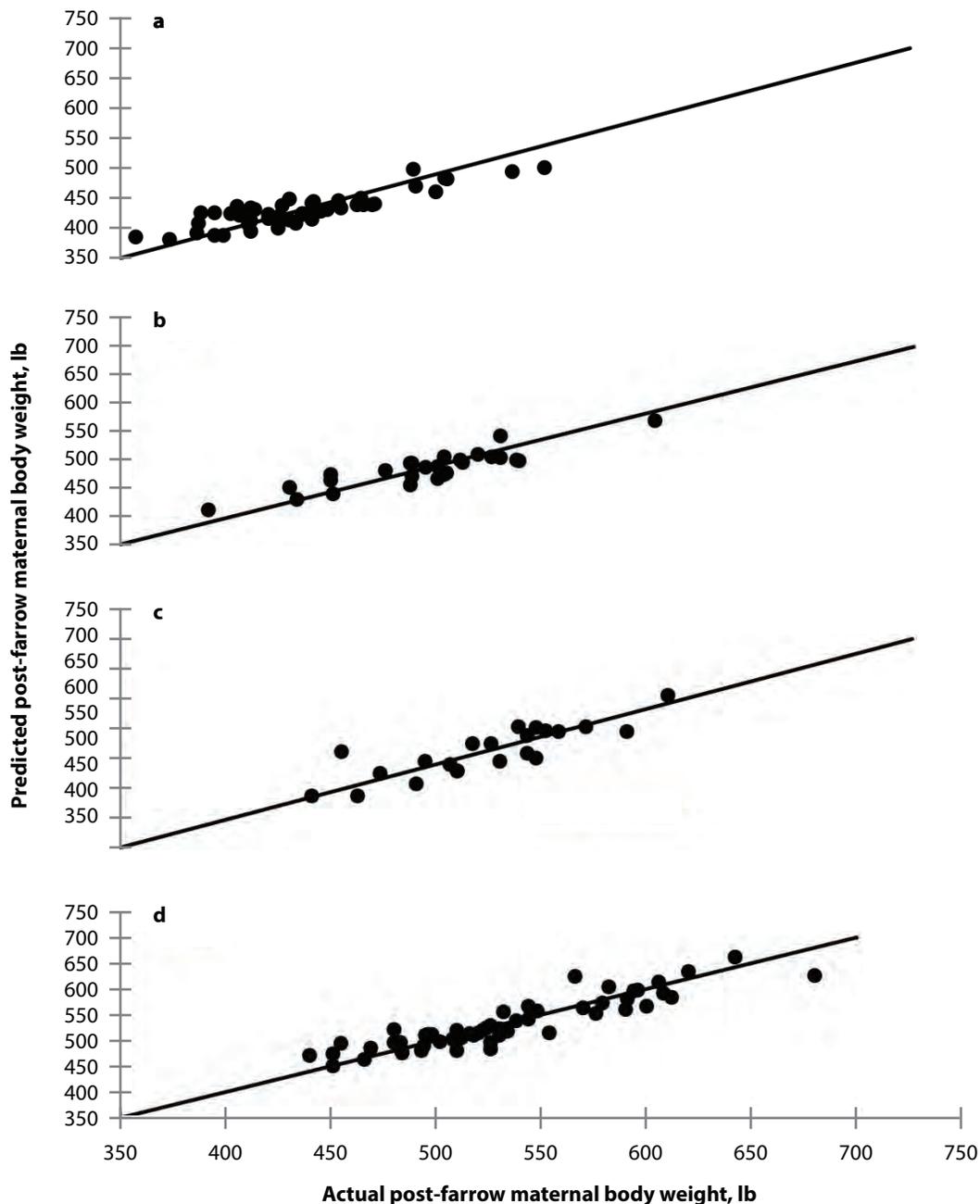
<sup>2</sup>Days pre- to post-farrow = Date post-farrow weight was obtained – date pre-farrow weight was obtained.

**Table 2. Descriptive statistics for data used for the prediction equation evaluation<sup>1</sup>**

Item	Mean	SD	Minimum	Maximum
Pre-farrow weight, lb	576.4	66.46	419	694
Actual post-farrow weight, lb	546.6	63.10	383	680
Estimated post-farrow weight, lb	549.85	69.00	378	687
Parity	2.8	1.40	1	7
Total born	14.8	2.94	7	22
Days pre- to post-farrow, d <sup>2</sup>	5.3	1.37	1	7

<sup>1</sup>A total of 332 females (PIC 1050) were used to validate the prediction equation to estimate post-farrow maternal weight.

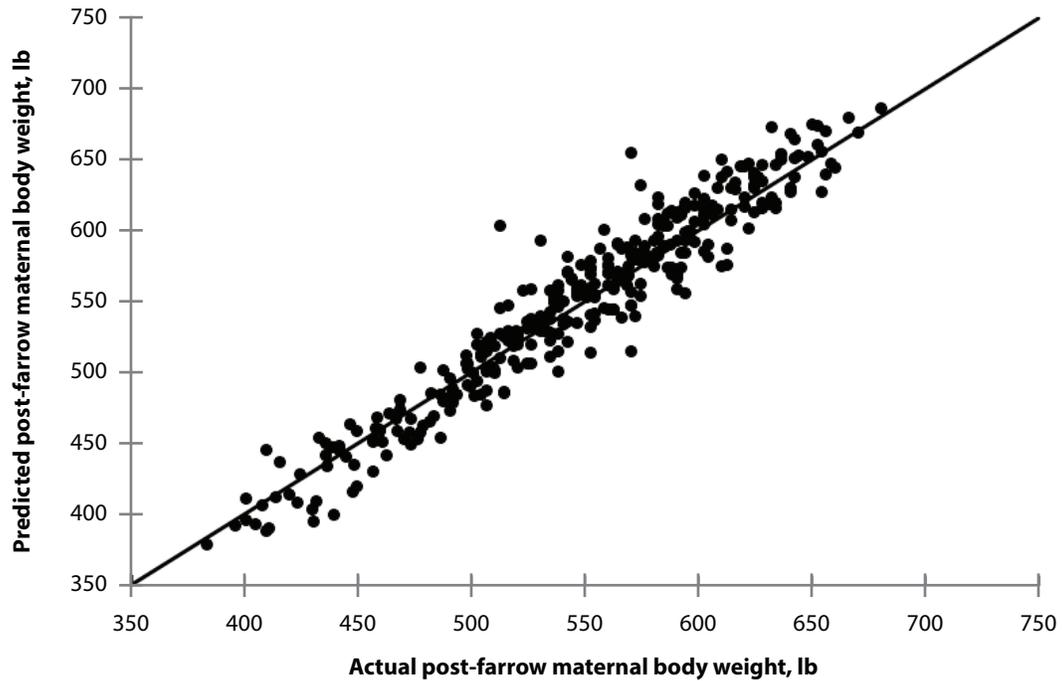
<sup>2</sup>Days pre- to post-farrow = Date post-farrow weight was obtained – date pre-farrow weight was obtained.



**Figure 1. Plot of actual maternal body weight (lb) vs. predicted maternal body weight (lb) relative to the line of equality for (a) parity 1, (b) parity 2, (c) parity 3, and (d) parity 4+ sows from the mixed model analysis. The optimum equations to predict maternal body weight were similar for all parities except for the intercept (b) and can be described as:**

$$\text{Post-farrow maternal body weight (lb)} = b + (0.897 \times \text{pre-farrow BW, lb}) - (1.118 \times \text{total born, n}) + (6.87 \times \text{days pre to post-farrow, d})$$

Where the intercept (b) for parities 1, 2, 3, and 4+ were -5.93, 5.15, 11.90, and 32.31, respectively.



**Figure 2. Comparison of actual and predicted maternal BW relative to the line of equality for sows in the validation experiment. The following equation was used for the prediction of maternal BW:**

$$\text{Post-farrow maternal body weight (lb)} = b + (0.897 \times \text{pre-farrow BW, lb}) - (1.118 \times \text{total born, n}) + (6.87 \times \text{days pre to post-farrow, d})$$

Where the intercept (b) for parities 1, 2, 3, and 4+ were -5.93, 5.15, 11.90, and 32.31, respectively.

## Effects of Lysine on Performance of Lactating Primiparous Sows

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### Summary

A total of 111 primiparous sows (Line 241; DNA, Columbus, NE) were used in a 21-d study to determine the effect of lysine (Lys) intake during lactation on sow and litter performance and subsequent reproductive performance of primiparous sows. At d 110 of gestation, sows were weighed and randomly assigned to treatment based on weight block. Dietary treatments consisted of increasing levels of standardized ileal digestible (SID) Lys (0.80, 0.95, 1.10, and 1.25% with other AA meeting or exceeding NRC [2012]<sup>2</sup> recommendations as a ratio to Lys). All other nutrients met or exceeded the NRC (2012) estimates. During the lactation period, there were no differences in ADFI or sow BW at d 0 or weaning, resulting in no differences in BW loss. However, backfat loss during lactation decreased (linear,  $P = 0.046$ ) as SID Lys increased. Regardless of treatment, there were no differences in litter weaning weight or litter gain from d 2 to weaning. In addition, no differences were observed for wean-to-estrus interval or the percentage of females bred by d 7 after weaning. However, d 30 conception rate increased (quadratic,  $P = 0.042$ ) as Lys increased up to 0.95% SID Lys, but then decreased as SID Lys reached 1.25%.

On the subsequent cycle, there was a tendency for decreased (quadratic,  $P = 0.054$ ) percentage born alive as Lys increased to 0.95% SID; however, percentage born alive increased thereafter. Reciprocally, percentage of mummies tended to increase (quadratic,  $P = 0.090$ ) with the greatest percentage mummies at 0.95% SID Lys. Overall, this study would suggest that in primiparous sows, there was no effect of increasing SID Lys above 0.80% on sow or litter performance. This study suggests that sow BF loss through lactation was decreased as SID Lys increased; however, little change on reproductive performance was observed. Additional research should be conducted with a larger group of sows housed under commercial conditions to confirm our findings.

Key words: lactation, lysine, reproduction, sows

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<sup>2</sup> National Research Council. *Nutrient Requirements of Swine: Eleventh Revised Edition*. Washington, DC: The National Academies Press, 2012. doi:10.17226/13298.

## Introduction

Lysine is the first limiting amino acid in corn-soybean meal-based swine diets. In order to maximize efficiency in all stages of production, the requirement of Lys needs to be determined. In lactation diets, nutrients need to be supplied to support both sow maintenance and litter growth. Inadequate nutrient intake during lactation can cause the sow to be catabolic and cause increased sow body protein mobilization.<sup>3</sup> Previous research has suggested a linear correlation between lactation Lys intake and litter weight gain.<sup>4</sup> However, this research was conducted with sows with performance levels that are far less than modern genetics. Because primiparous sows consume less feed, maternal growth accounts for a larger percentage of daily nutrient intakes. Thus, it is important to establish their Lys requirement separate from multiparous sows. Therefore, the objective of this study was to determine the effect of increasing dietary standardized ileal digestible (SID) Lys on the performance of lactating primiparous sows and their litters.

## Procedures

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State Swine Teaching and Research Farm in Manhattan, KS. Females were individually housed from d 0 to 110 of gestation and fed a common diet with 0.56% SID Lys according to body condition: thin, ideal, and fat females were fed 4.8, 4.5, and 4.2 lb/d, respectively.

A total of 111 primiparous females (Line 241, DNA, Columbus, NE) were used over 4 farrowing groups. At d 110 of gestation, females were weighed and moved to the farrowing house. Females were blocked by weight and expected farrowing date and randomly allotted to 1 of 4 treatments within those blocks. Dietary treatments were corn-soybean meal-based and consisted of increasing SID Lys (0.80, 0.95, 1.10, and 1.25%). Treatments were formed by increasing both crystalline lysine and soybean meal such that there was an increase in L-Lys HCl of 0.12% between each treatment with soybean meal increasing to meet the remainder of the SID Lys target for each treatment. Other feed-grade AA were added as required. All other nutrients and AA (as a ratio to Lys) met or exceeded the NRC (2012) requirement estimates (Table 1).

From d 110 to 113 of gestation, females were fed 6.0 lb/d of the gestation diet. Starting on d 113, sows received 6.0 lb/d of dietary treatment until farrowing. Postpartum, sows were allowed ad libitum feed intake with daily feed delivered and recorded by an electronic feeding system (Gestal Solo Feeders Jyga Technologies, Quebec City, Quebec, Canada). Feed intake was also recorded by weighing the amount placed in the feed hopper and the amount remaining at weaning. Sow BW and back fat depth (4 in from the midline of the last rib) were measured on d 0, d 10 post-farrowing, and at weaning, d 21. Cross fostering occurred irrespective of dietary treatment until 48 h postpartum in an attempt to equalize litter size (minimum of 10 pigs per litter for group 1 and 12

<sup>3</sup> Yang, H., J. E. Pettigrew, L. J. Johnston, G. C. Shurson, J. E. Wheaton, M. E. White, Y. Koketsu, A. F. Sower, and J. A. Rathmacher. 2000. Effects of dietary lysine intake during lactation on blood metabolites, hormones, and reproductive performance in primiparous sows. *Journal of Animal Science* 78:1001-1009. doi:/2000.7841001x.

<sup>4</sup> Johnston, L. J., J. E. Pettigrew, and J. W. Rust. 1993. Response of maternal-line sows to dietary protein concentration during lactation. *Journal of Animal Science*. 71:2151-2156. doi:10.2527/1993.7182151x.

pigs per litter for groups 2 to 4). Litters were weighed on d 2 and 10 post-farrowing and at weaning.

At weaning, sows were moved to a breeding barn, housed individually, and checked daily for signs of estrus using boar stimulus. The wean-to-estrus interval (WEI) was recorded when sows were first observed to show a positive response to the back-pressure test. Conception rate was recorded after ultrasound test at d 30.

After weaning, no dietary treatments were applied, and females were fed a common diet with 0.56% SID Lys according their body condition. Thin, ideal, and fat females were fed 4.8, 4.5, or 4.2 lb/d, respectively. Subsequent performance (total born, number born alive, birth weight, weaning weight) was collected from sows on their second parity.

Dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. A new batch was manufactured for each farrowing group. During bagging of the experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, 30th, and 35th bag, and these samples were pooled and used for AA and nutrient analysis.

Four samples (one per batch) per dietary treatment from the pooled samples were sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for proximate analysis, including CP, Ca, and P. Additionally, 4 samples (one per batch) per dietary treatment were sent for complete diet amino acid analysis (Ajinomoto Heartland Inc., Eddyville, IA; Table 2).

### ***Data Analysis***

Data were analyzed using generalized linear mixed models where dietary treatment was a fixed effect, with random effects of group and block. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC).

Sow ADFI, BW, BW loss, backfat loss, litter weight, litter gain, lactation length, litter size, subsequent total born and g/head/d total Lys consumed and were fitted assuming a normal distribution of the response variable. In these cases, residual assumptions were checked using studentized residuals and were found to be reasonably met.

Wean-to-estrus interval was fitted assuming negative binomial distribution. Females bred until d 7 after weaning and d 30 conception rate were fitted using a binary distribution. Subsequent litter performance variables, born alive, and percentage stillborns and mummies were all fitted using a binomial distribution. All results were considered significant at  $P \leq 0.05$  and marginally significant at  $0.05 < P \leq 0.10$ .

### **Results and Discussion**

Chemical analysis of DM, CP, Ca, P, and AA were similar to the formulated values (Table 2). The total Lys analysis of the low Lys diet was slightly higher than formulated. As a result, total Lys consumed in g/head/d increased (quadratic,  $P = 0.013$ ) as formulated SID Lys increased.

There were no differences between treatments in initial BW at d 110 of gestation, which validates the randomization of sows to treatments (Table 3). During the lactation period, there were no differences in sow BW at d 0 or weaning. Lactation ADFI did not differ between treatments; however, as expected, SID Lys intake per day increased (quadratic,  $P = 0.013$ ) with the highest daily SID Lys intake observed in sows fed 1.25% SID Lys. Backfat loss during lactation decreased (linear,  $P = 0.046$ ) as SID Lys increased; however, the difference in backfat loss between all treatments was less than 1 mm.

As expected, there were no differences observed in litter size or litter weight after equalization across treatments. Regardless of treatment, there were no differences in litter weaning weight and litter gain from d 2 to weaning.

There was no evidence for differences in lactation length between dietary treatments, which was expected. Additionally, no differences were observed for WEI between dietary treatments, even though it numerically decreased as SID Lys increased. There were no differences among dietary treatments for percentage of sows bred by d 7. However, there was an increase then decrease (quadratic,  $P = 0.042$ ) for d 30 conception rate as conception rate increased up to 0.95% SID Lys, but then was lowest in sows fed 1.25% SID Lys.

On the subsequent cycle, there was a tendency for decreased (quadratic,  $P = 0.054$ ) percentage born alive as Lys increased from 0.80 to 0.95% SID; however, percentage born alive increased from 0.95 to 1.25% SID Lys. Reciprocally, percentage of mummies increased then decreased (quadratic,  $P = 0.090$ ) as SID Lys increased with the greatest percentage mummies observed in sows fed 0.95% SID Lys.

A recent study evaluating SID Lys fed in lactation to primiparous sows showed a decrease in BW loss when SID Lys increased in the diet.<sup>5</sup> However, there was no effect on sow ADFI, WEI, subsequent reproduction, or litter performance, which would be in agreement with the current study. The current study would suggest there was no effect on sow or litter performance when Lys was fed beyond 0.80% SID lysine. This study suggests that sow BF loss through lactation was reduced as SID Lys increased; however, little change on reproductive performance was observed. One limitation of this experiment is that experimental treatments were only fed for 1 lactation period. Additional research should be conducted to determine the Lys requirement to maximize sow and litter performance. Another limitation is the few number of sows utilized in this study. Additional research should be conducted with a larger group of sows housed in commercial facilities in order to confirm our results.

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<sup>5</sup> Shi, M., Zang, J., Li, Z., Shi, C., Liu, L., Zhu, Z. and Li, D. 2015. Estimation of the optimal standardized ileal digestible lysine requirement for primiparous lactating sows fed diets supplemented with crystalline amino acids. *Journal of Animal Science*. 86: 891–896. doi:10.1111/asj.12377.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Standardized ileal digestible Lys, %			
	0.80	0.95	1.10	1.25
Corn	68.17	65.64	63.00	60.38
Soybean meal, 46.5% CP	25.58	27.89	30.21	32.49
Choice white grease	2.00	2.00	2.00	2.00
Limestone	1.30	1.28	1.28	1.25
Monocalcium P, 21% P	1.80	1.78	1.75	1.75
Salt	0.50	0.50	0.50	0.50
L-Lys-HCl	---	0.12	0.24	0.36
DL-Met	---	0.01	0.07	0.14
L-Thr	---	0.06	0.13	0.20
L-Trp	---	---	---	0.02
L-Val	---	0.09	0.18	0.28
Trace mineral premix	0.15	0.15	0.15	0.15
Sow vitamin premix	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	0.80	0.95	1.10	1.25
Ile:Lys	80	72	65	61
Leu:Lys	173	151	135	123
Met:Lys	32	29	31	34
Met and Cys:Lys	63	56	56	56
Thr:Lys	69	67	67	67
Trp:Lys	23	21	19	19
Val:Lys	89	87	87	87
Total Lys, %	0.93	1.08	1.24	1.40
NE, kcal/lb	1,133	1,129	1,125	1,121
CP, %	17.8	18.9	20.1	21.3
Ca, %	0.90	0.90	0.90	0.90
P, %	0.75	0.75	0.75	0.75
Available P, %	0.45	0.45	0.45	0.45

<sup>1</sup> Diets were fed from d 113 of gestation to weaning.

**Table 2. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item, %	Standardized ileal digestible Lys, %			
	0.80	0.95	1.10	1.25
DM	88.32	88.14	88.29	88.37
CP	17.99	18.68	20.01	21.40
Ca	1.03	1.05	1.08	1.09
P	0.77	0.75	0.79	0.79
Total AA, %				
Lys	1.01	1.12	1.26	1.43
Ile	0.72	0.74	0.78	0.85
Leu	1.55	1.48	1.64	1.74
Met	0.30	0.31	0.37	0.44
Met and Cys	0.63	0.64	0.71	0.81
Thr	0.70	0.77	0.86	0.97
Trp	0.22	0.22	0.24	0.27
Val	0.85	0.92	1.06	1.21
His	0.48	0.19	0.50	0.54
Phe	0.84	0.93	0.97	1.03

<sup>1</sup> Diet samples were collected from each batch of feed at manufacturing from every fifth bag. Crude protein and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc. (Chicago, IL). Dry matter, Ca, and P analyses were conducted on composite samples by Ward Laboratories (Kearney, NE).

**Table 3. Effects of increasing standardized ileal digestible (SID) lysine in lactation diets on sow and litter performance<sup>1</sup>**

Item	SID Lysine, %				SEM	P-value	
	0.80	0.95	1.10	1.25		Linear	Quadratic
BW, lb							
d 110	428.8	431.2	429.4	430.5	6.53	0.926	0.894
d 0	405.6	404.2	404.6	407.5	5.46	0.774	0.660
d 10	400.6	399.8	401.9	405.2	5.44	0.456	0.663
Wean	393.8	394.0	396.5	400.6	5.54	0.306	0.691
BW loss, lb							
d 0 to 10	-4.66	-4.29	-3.75	-2.65	2.012	0.473	0.854
d 10 to wean	-6.82	-5.83	-5.43	-4.58	1.934	0.393	0.970
d 0 to wean	-11.54	-10.20	-9.30	-7.24	2.892	0.281	0.897
ADFI							
d 0 to 10	10.24	9.67	10.00	10.22	0.273	0.713	0.108
d 10 to wean	13.99	13.80	13.67	13.88	0.386	0.743	0.573
d 0 to wean	11.00	10.40	10.74	10.98	0.287	0.653	0.115
Total Lys intake, g/d	50.2	52.9	61.2	71.2	1.53	0.001	0.013

*continued*

**Table 3. Effects of increasing standardized ileal digestible (SID) lysine in lactation diets on sow and litter performance<sup>1</sup>**

Item	SID Lysine, %				SEM	P-value	
	0.80	0.95	1.10	1.25		Linear	Quadratic
BF loss, mm							
d 0 to 10	-0.99	-1.62	-0.95	-1.09	0.249	0.549	0.306
d 10 to wean	-1.53	-0.91	-1.23	-0.57	0.253	0.046	0.934
d 0 to wean	-2.51	-2.53	-2.18	-1.65	0.329	0.046	0.410
Litter size, n							
d 2	13.06	13.17	13.05	13.22	0.167	0.662	0.819
d 10	13.06	13.17	13.05	13.22	0.167	0.662	0.819
Weaning	12.91	13.06	13.01	13.17	0.172	0.344	0.973
Litter weight, lb							
d 2	42.93	43.45	41.54	41.96	1.09	0.263	0.965
d 10	92.54	91.56	91.06	91.28	1.169	0.365	0.565
Weaning	152.14	150.79	148.80	153.40	2.117	0.387	0.473
Litter gain, lb							
d 2 to 10	50.10	49.12	48.62	48.84	1.170	0.365	0.565
d 10 to wean	59.61	59.18	57.77	59.10	1.257	0.515	0.451
d 2 to wean	109.70	108.34	106.36	107.90	2.117	0.387	0.473
Lactation length, d	18.7	18.8	18.6	18.4	0.30	0.010	0.450
Wean-to-estrus interval (WEI), d	5.00	4.91	4.97	4.61	0.45	0.691	0.800
Females bred by 7 d after weaning, %	96.0	96.0	96.4	100.0	3.91	0.978	0.979
d 30 conception rate, %	86.2	97.0	95.6	80.0	8.08	0.398	0.042
Subsequent performance <sup>2</sup>							
Total piglets born per sow farrowed, n	14.18	16.18	15.33	15.77	0.933	0.409	0.360
Born alive, %	94.2	89.8	91.0	93.7	2.05	0.955	0.054
Stillborn, %	5.0	7.2	7.0	5.0	1.73	0.998	0.193
Mummy, %	0.6	3.0	1.5	1.0	1.03	0.960	0.090

<sup>1</sup> A total of 111 primiparous sows (DNA 241, DNA Genetics) across 4 farrowing groups were used in a 21-d trial with 27 to 29 females per dietary treatment.

<sup>2</sup> Number of sows included for subsequent performance were 19, 22, 26, and 20 for dietary treatments of 0.80, 0.95, 1.10 and 1.25% SID Lys, respectively.

## Effects of Increasing Dietary Standardized Ileal Digestible Lysine on 15 to 24 lb Nursery Pigs<sup>1</sup>

*A.B. Clark, M.D. Tokach, J.M. DeRouchey, S.S. Dritz,<sup>2</sup> R.D. Goodband, J.C. Woodworth, and K.J. Touchette<sup>3</sup>*

### Summary

A total of 300 nursery pigs (PIC 327 × 1050, initially 14.8 lb BW) were used in a 28-d growth trial to evaluate the effects of increasing dietary standardized ileal digestible (SID) lysine (Lys) on nursery pig growth performance. Pigs were weaned at approximately 21 d of age and allotted to the pens according to BW and gender. A common starter diet was fed for 6 d, then pens were allotted to 1 of 6 dietary treatments in a completely randomized design. Experimental diets were fed for 14 d followed by a common diet for 14 d. The 6 dietary treatments were formulated to contain 1.10, 1.20, 1.30, 1.40, 1.50, and 1.60% SID Lys.

Increasing SID Lys resulted in improved (linear,  $P < 0.001$ ) ADG and F/G during d 0 to 14 when experimental diets were fed, with no differences observed in ADFI. For ADG, broken line linear (BLL) and quadratic polynomial (QP) models demonstrated similar fits, with maximum ADG at 1.45% and above 1.60% for BLL and QP models, respectively. Similar estimates were found when modeling feed efficiency.

In conclusion, this experiment determined that the SID Lys requirement for 15 to 24 lb nursery pigs was at least 1.45% SID Lys for both ADG and feed efficiency.

Key words: lysine, growth, nursery pigs, swine

### Introduction

Lysine is typically the first limiting amino acid in corn and soybean meal-based swine diets. Therefore, it is critical to establish the Lys requirement at each growth phase in order to allow the pig maximum growth potential and keep diets economical. Increasing crystalline amino acid usage to replace specialty protein sources and current statistical modeling capabilities has created a need for more research in amino acid requirements. Typically, essential amino acids are formulated in ratio to Lys. Thus, the Lys require-

<sup>1</sup> Appreciation is expressed to Ajinomoto Heartland, Inc., Chicago, IL, for partial financial support.

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ment must first be established to properly investigate next-limiting amino acid ratios. Therefore, the objective of this study was to determine the standardized ileal digestible (SID) Lys requirement for nursery pigs weighing approximately 15 to 25 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The trial was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS.

A total of 300 nursery pigs (PIC 327 × 1050) were used in the 28-d experiment. There were 10 replicate pens per treatment and 5 pigs per pen. Pigs were weaned at approximately 21 d of age (14.8 lb BW) and allotted to pens according to BW and gender. A common starter diet was fed for 6 d post-weaning. On d 6, pens were allotted to 1 of 6 dietary treatments in a completely randomized design. The six dietary treatments were formulated to contain 1.10, 1.20, 1.30, 1.40, 1.50, and 1.60% SID Lys and fed for 14 d followed by a common diet for 14 d. Both phases were fed in meal form. Diets were corn and soybean meal-based and contained 10% dried whey, with crystalline amino acids replacing corn. Extreme diets (1.10 and 1.60% SID Lys, Table 1) were manufactured first, then blended to create the intermediate treatments.

Pigs were weighed and feed disappearance was measured on d 0, 7, 14, 21, and 28. Each pen (5 × 5 ft) contained a 4-hole, dry, self-feeder and a nipple waterer to provide ad libitum access to feed and water. Samples of treatment diets were collected upon manufacturing at the feed mill and proximate analysis (Ward Laboratories, Inc., Kearney, NE) was conducted on composite samples. Additionally, experimental diet samples were submitted for amino acid analysis (Ajinomoto Heartland, Chicago, IL).

A base model where data were analyzed as a completely randomized design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit was initially evaluated. Results were considered significant at  $P \leq 0.05$ . The effect of SID Lys dose response on ADG and feed efficiency (modeled as gain to feed ratio; G:F) during the experimental period (d 0 to 14) were fit using PROC GLIMMIX and PROC NLMIXED according to procedures of Gonçalves et al. (2016).<sup>4</sup> Feed intake was not modeled, as there was no evidence of linear or quadratic effect of treatment. Dose response models evaluated were quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models. Heterogeneous variance was applied where appropriate. The Bayesian Information Criterion (BIC) was used to determine best fit, with a lower number indicating an improved fit. A decrease in BIC greater than 2 among models for a particular response criterion was considered a significant improvement in fit. Two outlier pens were removed from the data set, as they were greater than 2 standard deviations from the means.

<sup>4</sup> Gonçalves, M., N. Bello, S. Dritz, M. Tokach, J. DeRouchey, J. Woodworth, and R. Goodband. 2016. An update on modeling dose-response relationships: Accounting for correlated data structure and heterogeneous error variance in linear and nonlinear mixed models. *Journal of Animal Science*. 94(5): 1940-1950.

## Results and Discussion

Diet analysis matched formulated values (Table 2). Amino acids showed a step-wise increase in total Lys level as treatments increased in formulated SID Lys value.

During the experimental period (d 0 to 14), ADG and F/G improved (linear,  $P < 0.001$ ) as SID Lys increased, with no observed differences in ADFI (Table 3). There were no significant differences in ADG, ADFI, or F/G during the common period (d 14 to 28). During the overall period (d 0 to 28), ADG and F/G linearly improved ( $P < 0.001$ ) as SID Lys increased. Similarly, BW was improved in a linear manner ( $P < 0.001$ ) with increasing SID Lys on d 14 and 28.

Homogeneous variance was used for ADG models and heterogeneous variance was used for feed efficiency models. For ADG (Figure 1), the best fitting models were BLL and QP (BIC: 305.8 and 306.8, respectively). For the BLL, maximum ADG was achieved with a minimum of 1.45% SID Lys (95% CI: [1.31, 1.58%]). The QP  $[-0.403606 + 1.2932 \times (\text{SID Lys}) - 0.3721 \times (\text{SID Lys})^2]$  resulted in a maximum ADG above 1.60% SID Lys and 95% of maximum performance was achieved with 1.43% SID Lys. Feed efficiency (Figure 2), modeled as G:F, also had similar fitting models for the BLL and QP (BIC: 627.7 and 629.6, respectively). For the BLL, maximum G:F was achieved with a minimum of 1.45% SID Lys (95% CI: [1.35, 1.54%]). The QP  $[-0.3041 + 1.2081 \times (\text{SID Lys}) - 0.3485 \times (\text{SID Lys})^2]$  reported maximum G:F above 1.60% SID Lys and 95% of maximum performance was achieved with 1.41% SID Lys.

In conclusion, this experiment demonstrated that the Lys requirement for 15 to 25 lb nursery pigs was 1.45% SID Lys as reported by BLL models for both ADG and feed efficiency. Using QP models, the maximum was above 1.60%, with 95% of maximum performance achieved with 1.43% and 1.41% SID Lys, for ADG, and QP, respectively. Therefore, formulating nursery diets for pigs of this weight range to 1.45% SID Lys would allow for maximum growth responses in ADG and feed efficiency. This experiment was the first step in establishing a Lys requirement for subsequent trials evaluating other essential amino acids.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Formulated SID Lys, %		Common phase
	1.10	1.60	
Ingredient, %			
Corn	59.06	48.15	63.77
Soybean meal (48% CP)	26.89	27.05	32.86
Dried whey	10.00	10.00	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate (22% P)	1.60	1.50	1.10
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.25	0.55	0.3
DL-Met	0.13	0.33	0.12
L-Thr	0.10	0.26	0.12
L-Trp	0.02	0.06	--
L-Val	0.01	0.15	--
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
HP 300 <sup>2</sup>	0.00	10.00	--
Total	100.00	100.00	100.00
Calculated analysis <sup>3</sup>			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.10	1.60	1.22
Ile:Lys	64	57	63
Leu:Lys	133	109	129
Met:Lys	35	40	33
Met and Cys:Lys	60	59	57
Thr:Lys	65	65	63
Trp:lys	20.4	20.3	18.7
Val:Lys	70	70	69
Total Lys, %	1.23	1.77	1.37
ME, kcal/lb	1,477	1,498	1,484
NE, kcal/lb	1,101	1,092	1,092
SID Lys:ME, g/Mcal	3.38	4.84	3.73
SID Lys:NE, g/Mcal	4.57	7.44	5.16
CP, %	19.3	24.7	21.4
Ca, %	0.82	0.83	0.70
P, %	0.76	0.79	0.64
Available P, %	0.48	0.48	0.41

<sup>1</sup>Treatments 1.10% and 1.60% SID Lys were manufactured and blended at the feed mill to create the intermediate levels of 1.20%, 1.30%, 1.40%, and 1.50% SID Lys.

<sup>2</sup>Hamlet Protein, Findley, OH.

<sup>3</sup>NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

**Table 2. Chemical analysis of experimental diets (as-fed basis)<sup>1</sup>**

Item	Formulated standardized ileal digestible (SID) Lys, % <sup>2</sup>					
	1.10	1.20	1.30	1.40	1.50	1.60
Proximate analysis, % <sup>3</sup>						
DM	88.77	88.24	88.81	87.35	89.18	89.22
CP	20.6	20.9	21.6	23.0	23.4	24.4
Crude fiber	1.8	1.7	2.1	1.9	1.9	2.2
Ether extract	2.5	2.2	2.4	2.4	2.3	2.4
Ash	5.05	5.58	5.31	5.60	5.81	5.52
Amino acid analysis, % <sup>4</sup>						
Lys	1.26	1.38	1.42	1.52	1.60	1.75
Ile	0.83	0.86	0.91	0.94	0.96	1.02
Leu	1.76	1.76	1.83	1.88	1.93	1.98
Met	0.40	0.47	0.48	0.51	0.54	0.65
Met + Cys	0.75	0.81	0.84	0.88	0.92	1.04
Thr	0.80	0.85	0.92	1.00	1.02	1.12
Trp	0.25	0.26	0.28	0.30	0.32	0.35
Val	0.91	0.95	1.03	1.08	1.12	1.22
His	0.49	0.52	0.52	0.56	0.58	0.60
Phe	0.95	0.98	1.03	1.06	1.11	1.15

<sup>1</sup>Diet samples were collected at the feed mill after manufacturing.

<sup>2</sup>Low (1.10% SID Lys) and high (1.60% SID Lys) diets were blended at the feed mill to create the intermediate treatments.

<sup>3</sup>Composite samples were submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

<sup>4</sup>Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for amino acid analysis.

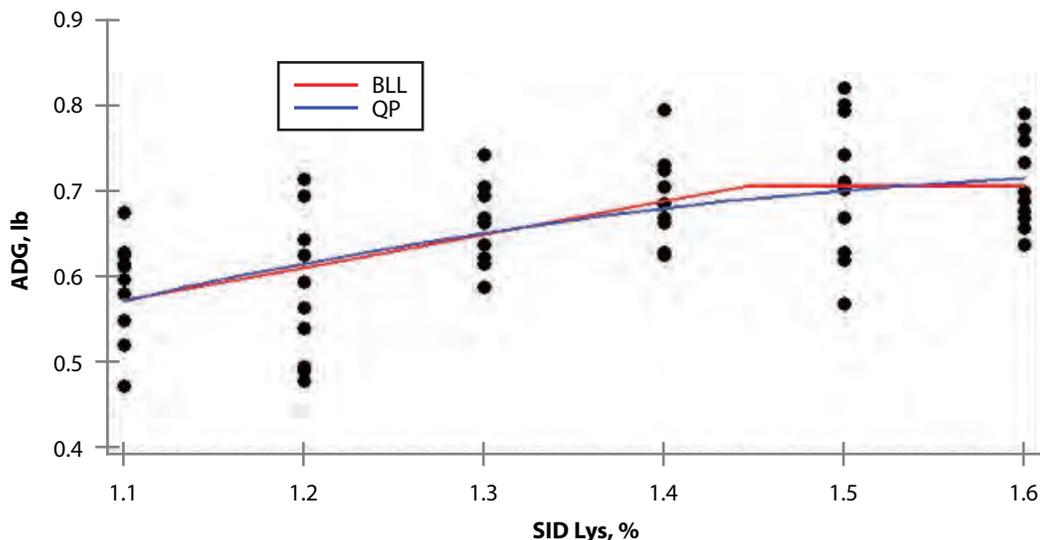
**Table 3. Effects of standardized ileal digestible (SID) Lys on nursery pig growth performance<sup>1,2</sup>**

Item	Formulated standardized ileal digestible (SID) Lys, % <sup>3</sup>						SEM	Probability, <i>P</i> <	
	1.10	1.20	1.30	1.40	1.50	1.60		Linear	Quadratic
Phase 1 (d 0 to 14)									
ADG, lb	0.58	0.58	0.66	0.69	0.70	0.71	0.022	0.001	0.278
ADFI, lb	0.95	0.92	0.98	0.97	0.97	0.97	0.032	0.336	0.835
F/G	1.64	1.60	1.50	1.40	1.39	1.38	0.040	0.001	0.136
Phase 2 (d 14 to 28)									
ADG, lb	1.24	1.25	1.28	1.22	1.27	1.28	0.029	0.391	0.652
ADFI, lb	1.95	1.96	2.02	1.93	2.00	2.04	0.040	0.154	0.558
F/G	1.57	1.57	1.58	1.58	1.57	1.59	0.023	0.540	0.966
Overall (d 0 to 28)									
ADG, lb	0.91	0.92	0.97	0.96	0.99	0.99	0.022	0.001	0.797
ADFI, lb	1.45	1.44	1.50	1.45	1.49	1.51	0.033	0.180	0.799
F/G	1.59	1.61	1.55	1.52	1.51	1.52	0.021	0.001	0.336
BW, lb									
d 0	14.8	14.8	14.9	14.8	14.8	14.8	0.133	0.952	0.721
d 14	23.0	22.8	24.1	24.5	24.6	24.7	0.338	0.001	0.263
d 28	40.4	40.4	42.0	41.6	42.5	42.7	0.633	0.001	0.758

<sup>1</sup> A total of 300 nursery pigs (PIC 327 × 1050, initially 14.8 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets.

<sup>2</sup> Experimental diets were fed from d 0 to 14 and a common diet was fed from d 14 to 28.

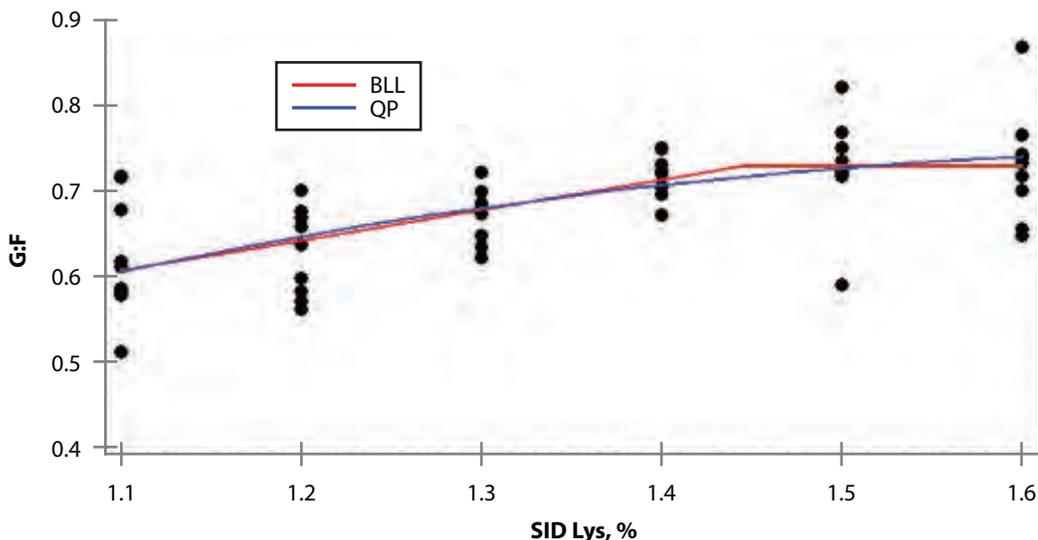
<sup>3</sup> Low (1.10% SID Lys) and high (1.60% SID Lys) diets were blended upon manufacturing at the feed mill to create the 1.20, 1.30, 1.40, and 1.50% SID Lys dietary treatments.



**Figure 1. The standardized ileal digestible (SID) Lys dose response curve for ADG in nursery pigs.**

**ADG** **BLL** 1.45% (95% CI: 1.31, 1.58)  
 (BIC = 305.8)  
**QP** Maximum: >1.60%  
 95% Maximum: 1.43%  
 $[-0.403606 + 1.2932 \times (\text{SID Lys}) - 0.3721 \times (\text{SID Lys})^2]$   
 (BIC = 306.8)

A total of 300 nursery pigs (PIC 327 × 1050, initially 14.8 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to characterize the SID Lys dose response curve. The BLL and QP models were the best fitting models based on Bayesian Information Criterion (BIC).



**Figure 2. The standardized ileal digestible (SID) Lys dose response curve for feed efficiency (G:F) in nursery pigs.**

**G:F**    **QP** Maximum: >1.60%  
 95% Maximum: 1.41%  
 $[-0.3041 + 1.2081 \times (\text{SID Lys}) - 0.3485 \times (\text{SID Lys})^2]$   
 (BIC = 629.6)  
**BLL** 1.45% (95% CI: 1.35, 1.54)  
 (BIC = 627.7)

A total of 300 nursery pigs (PIC 327 × 1050, initially 14.8 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 10 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to characterize the SID Lys dose response curve. The BLL and QP models were the best fitting models based on Bayesian Information Criterion (BIC).

## Effects of Dietary Standardized Ileal Digestible Valine:Lysine Ratio on 14 to 22 lb Nursery Pigs<sup>1</sup>

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### Summary

A total of 280 nursery pigs (PIC 327 × 1050; initially 14.4 lb BW) were used in a 28-d growth trial to evaluate the effects of increasing dietary standardized ileal digestible (SID) Valine:Lysine (Val:Lys) ratio on nursery pig growth performance. Pigs were weaned at approximately 21 d of age and allotted to pens according to BW and gender. A common starter diet was fed for 5 d, and then pens were allotted to 1 of 7 dietary treatments in a randomized complete block design according to BW. Experimental diets were fed for 14 d, which included SID valine concentrations of 50, 57, 63, 68, 73, 78, and 85% of Lys. Then pigs were fed a common Phase 3 diet for 14 d.

From d 0 to 14, when experimental diets were fed, ADG, ADFI, and F/G improved (quadratic,  $P < 0.036$ ) as SID Val:Lys ratio increased. For ADG, the best-fitting model was the broken line linear (BLL). This model resulted in a maximum ADG to be achieved when feeding a minimum of 62.9% SID Val:Lys ratio. For ADFI, the quadratic polynomial (QP) was the best fitting model, predicting maximum feed intake at 73.7% SID Val:Lys ratio and 99% of maximum performance achieved with 68.0% SID Val:Lys ratio. For feed efficiency, modeled as G:F, the best-fitting model was the QP, estimating maximum G:F at 71.7% SID Val:Lys ratio. In conclusion, this experiment demonstrated that the SID valine requirement for 14 to 22 lb nursery pigs ranged from 62.9 to 73.7% of Lys depending on the response criteria modeled.

Key words: valine, growth, nursery pigs, swine

### Introduction

Inclusion of dietary crystalline amino acids is a common practice in the swine industry. This is done to meet specific amino acid requirements while reducing feed cost and environmental impact. Additionally, amino acids are often expressed in relation to lysine to develop the most efficient diet formulations. A previous experiment conducted

<sup>1</sup> Appreciation is expressed to Ajinomoto Heartland, Inc. (Chicago, IL) for partial financial support.

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<sup>3</sup> Ajinomoto Heartland, Inc. (Chicago, IL).

at Kansas State University validated that the lysine requirement for 15 to 25 lb pigs was 1.45% SID lysine. Therefore, our next step was to determine the appropriate SID Val:Lys ratio for pigs in this weight range.

The NRC (2012)<sup>4</sup> estimates that the valine requirement for approximately 15 to 25 lb pigs is 63.7% SID Val:Lys, while Nemechek et. al (2014)<sup>5</sup> determined that 65% SID Val:Lys was necessary for optimal growth of 15 to 25 lb pigs. However, pigs in this experiment were fed at the Lys requirement for ADG, which may underestimate the Val:Lys requirement. Therefore, the objective of this study was to determine the SID Val:Lys requirement for nursery pigs weighing approximately 14 to 22 lb when fed marginally below their dietary lysine requirement.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The trial was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Corn, soybean meal, and whey were analyzed for amino acid content prior to diet formulation (Ajinomoto Heartland, Chicago, IL).

A total of 280 nursery pigs (PIC 327 × 1050; 14.4 lb BW) were used in a 28-d experiment. There were 8 replicate pens per treatment and 5 pigs per pen. Pigs were weaned at approximately 21 d of age and allotted to pens according to BW and gender. A common Phase 1 starter diet was fed 5 d post-weaning. On d 5 after weaning, pens were allotted to 1 of 7 dietary treatments by BW in a randomized complete block design. The 7 dietary treatments were formulated to contain SID Val at 50, 57, 63, 68, 73, 78, and 85% of Lys. Treatment diets were fed for 14 d followed by a common Phase 3 diet fed for 14 d. Both the experimental and common diets were fed in meal form.

Pigs were weighed and feed disappearance was measured on d 0, 7, 14, 21, and 28. Each pen (4 × 5 ft) contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Samples of treatment diets were collected upon manufacturing at the feed mill. Proximate analysis was conducted on composite samples (Ward Laboratories, Inc., Kearney, NE). In addition, experimental diet samples were submitted for amino acid analysis (Ajinomoto Heartland, Chicago, IL).

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and initial BW as a covariate. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ . The effect of SID Val:Lys ratio dose response on ADG, ADFI, and feed efficiency (modeled as gain to feed ratio; G:F) during the experimental period (d 0 to 14) were fit using PROC GLIMMIX and PROC NLMIXED accord-

<sup>4</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

<sup>5</sup> Nemechek, J. E.; Tokach, M. D.; Dritz, S. S.; Goodband, R. D.; DeRouchey, J. M. 2014. Evaluation of standardized ileal digestible valine:lysine, total lysine:crude protein, and replacing fish meal, meat and bone meal, and poultry byproduct meal with crystalline amino acids on growth performance of nursery pigs from seven to twelve kilograms. *Journal of Animal Science*. 2014.92:1548–1561.

ing to procedures of Gonçalves et al. (2016).<sup>6</sup> For ADFI, block was removed from the model as it did not contribute to model fit. Dose response models evaluated were quadratic (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models. Bayesian Information Criterion (BIC) was used to determine best fit, with a lower number indicating an improved fit. A decrease in BIC greater than 2.0 among models for a particular response criterion was considered an improved fit.

## Results and Discussion

The calculated experimental diet (Table 1) composition was similar to analyzed values (Table 2). Additionally, amino acid analysis matched intended levels, with lysine remaining constant while valine increased in a step-wise manner.

From d 0 to 14, when experimental diets were fed, ADG, ADFI, and F/G improved (quadratic,  $P < 0.036$ ) as SID Val:Lys ratio increased (Table 3). During the common Phase (d 14 to 28), ADFI increased and F/G became poorer (linear,  $P < 0.028$ ) in pigs previously fed diets containing increasing SID Val:Lys ratio. During the overall period (d 0 to 28), ADG marginally improved (quadratic,  $P = 0.089$ ), while ADFI increased (linear,  $P = 0.006$ ) and F/G was marginally poorer (linear,  $P = 0.094$ ) as SID Val:Lys ratio increased. Similarly, BW was increased (quadratic,  $P = 0.001$ ) on d 14 and marginally increased (linear,  $P = 0.057$ ) with increasing SID Lys on d 28.

Heterogeneous variance was used for ADG models and homogeneous variance was used for ADFI and feed efficiency models. For ADG (Figure 1), the BLL was the best fit and the maximum ADG was obtained with a minimum of 62.9% SID Val:Lys ratio (95% CI: [52.2, 73.7%]). For ADFI, (Figure 2) the QP  $[-0.5740219 + 0.039020944 \times (\text{Val:Lys}) - 0.000264771 \times (\text{Val:Lys})^2]$  was the best fitting model, predicting maximum feed intake at 73.7% SID Val:Lys ratio and 99% of maximum performance achieved with 68.0% SID Val:Lys ratio. Feed efficiency (Figure 3), modeled as G:F, found that the best fit was the QP  $[0.010294 + 0.017526 \times (\text{Val:Lys}) - 0.000122 \times (\text{Val:Lys})^2]$ . This model reported a maximum G:F at 71.7% SID Val:Lys ratio and 99% of maximum performance achieved with 64.4% SID Val:Lys.

As previously stated, Nemechek et al. (2014) determined that 65% SID Val:Lys ratio was necessary for optimal growth in pigs of similar weight range to those used in this study. This coincides with the current trial, however multiple models in our trial provide a range of estimated requirements depending on model and response criteria. Using similar modeling techniques, Gonçalves et al. (2015)<sup>7</sup> determined that the ideal SID Val:Lys ratio for pigs weighing 55 to 100 lb was 71.0% for feed efficiency and 74.0% for ADG. Although this result is for heavier pigs, it is very similar to the feed efficiency result determined in the present study. In conclusion, this experiment demonstrated that

<sup>6</sup> Gonçalves, M., N. Bello, S. Dritz, M. Tokach, J. DeRouche, J. Woodworth, and R. Goodband. 2016. An update on modeling dose-response relationships: Accounting for correlated data structure and heterogeneous error variance in linear and nonlinear mixed models. *Journal of Animal Science*. 94(5): 1940-1950.

<sup>7</sup> Gonçalves, M. A.; Jacquez, J.; Tokach, M. D.; Dritz, S. S.; Touchette, K. J.; DeRouche, J. M.; Woodworth, J. C.; and Goodband, R. D. (2015). Effects of Standardized Ileal Digestible Valine:Lysine Ratio on the Growth Performance and Economics of Finishing Pigs from 55 to 100 lb. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

the SID Val:Lys ratio requirement for approximately 14 to 22 lb nursery pigs ranged from 62.9 to 73.7% depending on the response criteria modeled.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Formulated % SID Val:Lys Ratio		
	50	85	Common phase
Ingredient, %			
Corn	62.97	62.50	63.77
Soybean meal (48% CP)	22.07	22.11	32.86
Dried whey	10.00	10.00	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate (22% P)	1.65	1.65	1.10
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.63	0.63	0.3
DL-Met	0.27	0.27	0.12
L-Thr	0.29	0.29	0.12
L-Trp	0.08	0.08	--
L-Val	0.00	0.44	--
L-Ile	0.10	0.10	--
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.24	1.24	1.22
Ile:Lys	57	57	63
Leu:Lys	110	110	129
Met:Lys	40	40	33
Met and Cys:Lys	60	60	57
Thr:Lys	66	66	63
Trp:Lys	20.1	20.1	18.7
Val:Lys	50	85	69
Total Lys, %	1.36	1.36	1.37
ME, kcal/lb	1,492	1,496	1,484
NE, kcal/lb	1,101	1,092	1,092
SID Lys:ME, g/Mcal	3.75	3.74	3.73
SID Lys:NE, g/Mcal	5.09	5.08	5.16
CP, %	17.6	17.9	21.4
Ca, %	0.82	0.82	0.70
P, %	0.73	0.73	0.64
Available P, %	0.49	0.49	0.41

<sup>1</sup>Treatments 50% and 85% SID Val:Lys were manufactured and blended at the feed mill to create the intermediate levels of 57, 63, 68, 73, and 78 % SID Val:Lys.

**Table 2. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Formulated % SID Val:Lys ratio <sup>2</sup>						
	50	57	63	68	73	78	85
Proximate analysis, % <sup>3</sup>							
DM	89.84	90.16	90.37	90.24	90.35	90.06	90.24
CP	17.0	18.7	17.6	18.0	18.0	19.3	17.6
Crude fiber	2.0	1.7	1.7	1.7	1.2	2.0	1.8
Ether extract	2.6	2.2	2.2	2.4	2.2	2.3	2.2
Ash	5.25	5.58	5.26	5.08	5.17	5.14	5.14
Amino acid analysis, % <sup>4</sup>							
Lys	1.32	1.33	1.37	1.35	1.35	1.33	1.34
Ile	0.76	0.78	0.77	0.77	0.78	0.87	0.80
Leu	1.56	1.54	1.54	1.51	1.55	1.61	1.59
Met	0.46	0.50	0.50	0.48	0.49	0.46	0.48
Met + Cys	0.73	0.77	0.77	0.74	0.78	0.75	0.77
Thr	0.92	0.89	0.92	0.89	0.90	0.94	0.96
Trp	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Val	0.78	0.84	0.88	0.92	0.99	1.04	1.10
His	0.43	0.42	0.41	0.41	0.42	0.44	0.43
Phe	0.83	0.92	0.82	0.80	0.83	0.86	0.84

<sup>1</sup>Treatment diet samples were collected at the feed mill after manufacturing.

<sup>2</sup>Low (50% SID Val:Lys) and high (85% SID Val:Lys) diets were blended at the feed mill to create the intermediate treatments.

<sup>3</sup>Composite samples were submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

<sup>4</sup>Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for amino acid analysis.

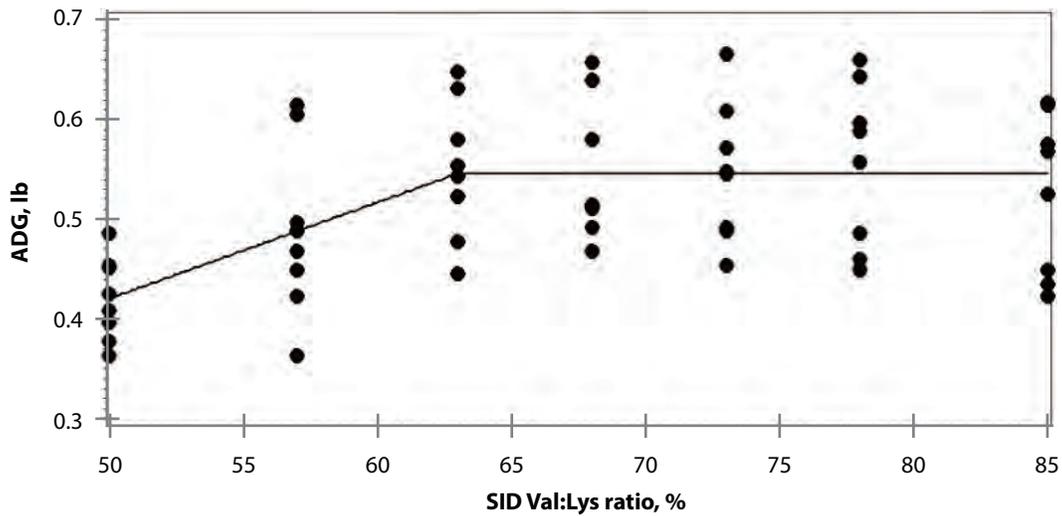
**Table 3. Effects of standardized ileal digestible Val:Lys ratio on nursery pig growth performance<sup>1</sup>**

Item	Formulated % SID Val:Lys <sup>2</sup>							SEM	Probability, <i>P</i> <	
	50	57	63	68	73	78	85		Linear	Quadratic
Phase 1 (d 0 to 14) <sup>3</sup>										
ADG, lb	0.42	0.49	0.55	0.55	0.55	0.55	0.52	0.025	0.001	0.001
ADFI, lb	0.73	0.80	0.87	0.86	0.89	0.86	0.85	0.038	0.012	0.030
F/G	1.74	1.65	1.60	1.56	1.63	1.56	1.64	0.050	0.084	0.036
Phase 2 (d 14 to 28)										
ADG, lb	1.19	1.17	1.14	1.27	1.15	1.17	1.19	0.034	0.992	0.945
ADFI, lb	1.82	1.80	1.82	1.94	1.87	1.91	1.93	0.051	0.028	0.965
F/G	1.53	1.54	1.61	1.53	1.63	1.64	1.63	0.025	0.001	0.945
Overall (d 0 to 28)										
ADG, lb	0.81	0.83	0.84	0.91	0.85	0.86	0.86	0.024	0.067	0.089
ADFI, lb	1.28	1.30	1.34	1.40	1.38	1.38	1.39	0.038	0.006	0.266
F/G	1.58	1.57	1.59	1.54	1.63	1.61	1.63	0.026	0.094	0.288
BW, lb										
d 14	20.3	21.2	22.1	22.1	22.0	22.2	21.8	0.35	0.001	0.001
d 28	36.9	37.6	37.7	39.8	38.1	38.5	38.5	0.760	0.057	0.146

<sup>1</sup> A total of 280 nursery pigs (PIC 327 × 1050, initially 14.4 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then placed on test. An initial (d 0) BW of 14.4 lb was used as a covariate.

<sup>2</sup> Low (50% SID Val:Lys ratio) and high (85% SID Val:Lys ratio) diets were blended upon manufacturing at the feed mill to create the 57, 63, 68, 73, and 78% SID Val:Lys ratio dietary treatments.

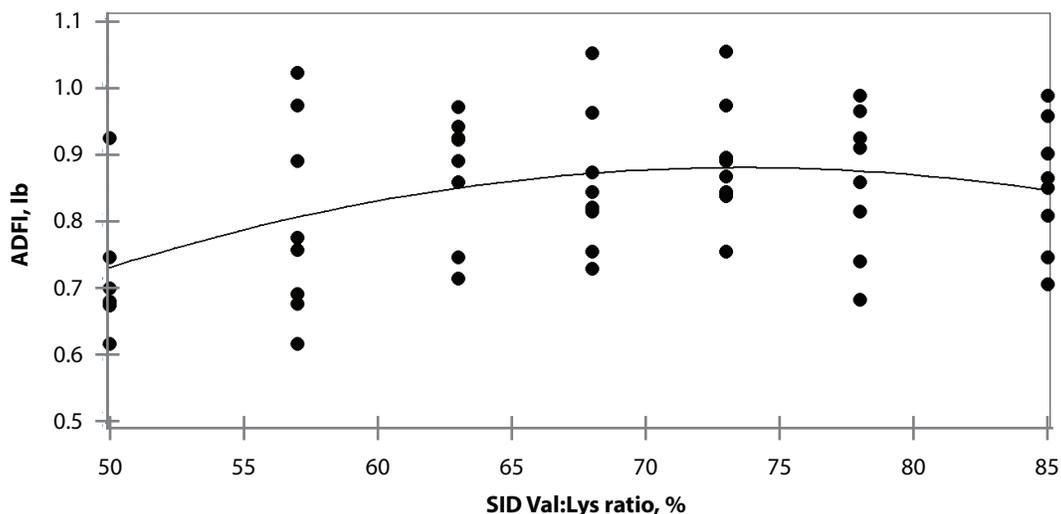
<sup>3</sup> Experimental diets were fed from d 0 to 14 and a common Phase 3 diet was fed from d 14 to 28.



**Figure 1. Estimated standardized ileal digestible Val:Lys ratio requirement to maximize ADG for nursery pigs.**

**ADG** **BLL** 62.9% (95% CI: 52.2, 73.7)

A total of 280 nursery pigs (PIC 327 × 1050, initially 14.4 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then placed on test. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys ratio level to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.



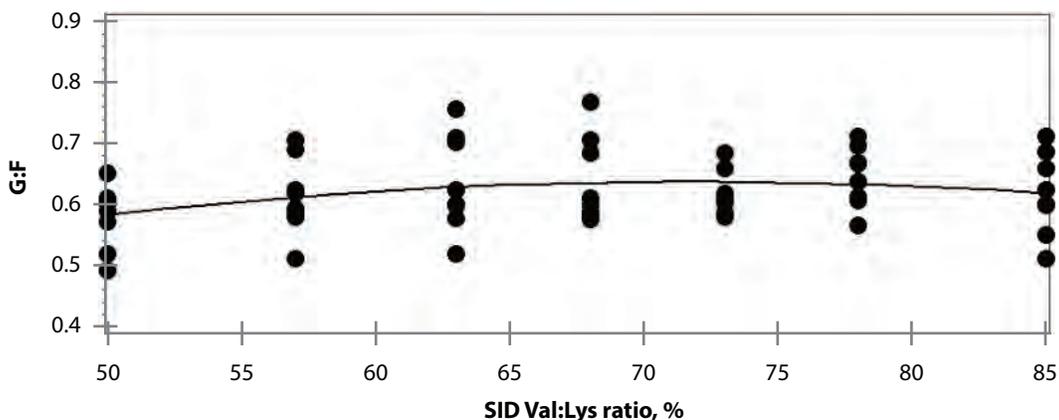
**Figure 2. Estimated standardized ileal digestible Val:Lys ratio requirement to maximize ADFI for nursery pigs.**

ADFI QP Maximum: 73.7%

99% Maximum: 68.0%

$$[-0.5740219 + 0.039020944 \times (\text{Val:Lys}) - 0.000264771 \times (\text{Val:Lys})^2]$$

A total of 280 nursery pigs (PIC 327 × 1050, initially 14.4 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then placed on test. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys ratio level to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.



**Figure 3. Estimated standardized ileal digestible Val:Lys ratio requirement to maximize G:F for nursery pigs.**

**G:F** QP Maximum: 71.7%

99% Maximum: 64.6%

$$[0.010294 + 0.017526 \times (\text{Val:Lys}) - 0.000122 \times (\text{Val:Lys})^2]$$

A total of 280 nursery pigs (PIC 327 × 1050, initially 14.4 lb BW) were used in a 28-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 5 d post-weaning, then placed on test. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit to estimate SID Val:Lys level to maximize G:F, as well as SID Val:Lys level to achieve 99% of maximum G:F using the QP model. Bayesian Information Criterion (BIC) was used to determine the best fitting models; a lower value indicates a better fit to the data.

## Effects of Dietary Standardized Ileal Digestible Isoleucine:Lysine Ratio on Nursery Pig Performance<sup>1</sup>

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### Summary

A total of 560 nursery pigs were used in 2 experiments to evaluate the effects of increasing dietary standardized ileal digestible (SID) Isoleucine:Lysine (Ile:Lys) ratio on growth performance. In Exp. 1, 280 pigs (PIC 327 × 1050, initially 14.9 lb BW) were fed experimental diets for 12 d with 8 replications and 5 pigs per pen. In Exp. 2, 280 pigs (DNA Genetics Line 600 × Line 241, initially 13.3 lb BW) were fed experimental diets for 18 d with 8 replications and 5 pigs per pen. In both experiments, pens were allotted to 1 of 7 dietary treatments in a randomized complete block design. The 7 dietary treatments were 40, 44, 48, 52, 54, 58, and 63% SID Ile:Lys ratio. After the experimental diet feeding period, a common diet was fed for 14 d. Diets in both phases were fed in meal form.

For Exp. 1, from d 0 to 12 when experimental diets were fed, ADG and ADFI improved (ADG, linear,  $P < 0.001$ ; and ADFI, quadratic,  $P < 0.017$ ) and F/G became poorer (quadratic,  $P < 0.041$ ) as SID Ile:Lys ratio increased. For ADG, the quadratic (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models reported maximum ADG at 64.7, 52.0, and 52.0% SID Ile:Lys ratio, respectively. For ADFI, the BLL breakpoint occurred at 50.6% and the QP predicted maximum ADFI at 56.2% SID Ile:Lys ratio.

In Exp. 2, from d 0 to 18 when experimental diets were fed, ADG and ADFI improved (quadratic,  $P < 0.009$ ) with no significant differences for F/G as SID Ile:Lys ratio increased. For ADG, the BLL and QP had similar fit with breakpoints/maximums occurring at 51.8% SID Ile:Lys ratio and 58.3% SID Ile:Lys ratio, respectively. For ADFI, the QP reported maximum ADFI at 57.2% SID Ile:Lys ratio and the BLQ breakpoint occurred at 52.0% SID Ile:Lys.

In summary, these experiments demonstrate that the SID Ile requirement for 15 to 25 lb nursery pigs is approximately 52% of Lys for ADG and ADFI using broken line mod-

<sup>1</sup> Appreciation is expressed to Ajinomoto Heartland, Inc. (Chicago, IL) for partial financial support.

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els and can be as high as 64% of Lys using quadratic models. A slight quadratic effect was observed in feed efficiency for Exp. 1, however in Exp. 2, there were no appreciable differences detected in F/G. The Ile requirement for 15 to 25 lb pigs was found to be similar to NRC (2012)<sup>4</sup> requirement estimates.

Key words: isoleucine, growth, nursery pigs, swine

## Introduction

The inclusion of crystalline amino acids in swine diets is an effective strategy to not only meet specific nutritional requirements, but also reduce diet cost and environmental impact. Typically, amino acids are expressed in ratio to lysine (Lys) for diet formulation process. Thus, it is important to evaluate essential amino acids in a Lys deficient scenario to appropriately identify the requirement of the essential amino acid of interest. A previous experiment conducted at Kansas State University demonstrated that the Lys requirement for 15 to 25 lb pigs was 1.45% SID Lys. Previously, we have determined the Thr, Met, Trp, and Val to Lys ratios for pigs from 15 to 25 lb. Therefore, our next step was to determine the appropriate SID Ile:Lys ratio for pigs in this stage of growth.

Mixed model statistical methods have recently been adapted in which multiple models are applied to the data and best fit objectively identified. The NRC (2012) estimates that the Ile requirement for approximately 15 to 25 lb pigs is 51.1% of Lys. Therefore, the objective of this study was to determine the SID Ile:Lys requirement for nursery pigs weighing approximately 15 to 25 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. Two experiments were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS, where each pen (Exp. 1: 5 ft × 5 ft; Exp. 2: 4 ft × 5 ft) contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS.

Diets in both experiments were corn and soybean meal-based with crystalline amino acids increasing to replace soybean meal. Diets contained 10% dried whey and 10% field peas. The field peas were used to lower the Ile:Lys ratio to allow titration of the requirement. The 7 dietary treatments in both experiments were 40, 44, 48, 52, 54, 58, and 63% SID Ile:Lys ratio. Upon manufacturing at the feed mill, the low (40%) and high (63%) treatment diets were blended to create the intermediate diets. Corn, soybean meal, field peas and dried whey were analyzed for AA content prior to diet formulation (Table 1). A new field pea batch and analysis prior to Exp. 2 called for a minor adjustment to Exp. 2 diets by decreasing crystalline amino acids slightly.

## Experiment 1

A total of 280 nursery pigs (PIC 327 × 1050; 14.9 lb BW) were used in a 26-d experiment. There were 8 replicate pens per treatment and 5 pigs per pen. Pigs were weaned

<sup>4</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

at approximately 21 d of age and allotted to the nursery according to BW and gender. A common starter diet was fed for 6 d post-weaning. On d 6, pens were allotted to 1 of 7 dietary treatments by BW and location in a randomized complete block design. Treatment diets were fed for 12 d followed by a common diet for 14 d. Both phases were fed in meal form. Pigs were weighed and feed disappearance was measured on d 0, 7, 12, 19, and 26.

### *Experiment 2*

A total of 280 nursery pigs (DNA Genetics Line 600 × Line 241, initially 13.3 lb BW) were used in a 32-d experiment. There were 8 replicate pens per treatment and 5 pigs per pen. Pigs were weaned at approximately 20 d of age and allotted to the nursery according to BW, gender, and age. One replication was fed a common starter diet for 3 d due to heavier weaning BW and age, and then placed on experimental diets. The remaining 7 replications were fed a common starter diet for 6 d post-weaning before being placed on treatment diets. On d 3 for the first replication and d 6 for the remaining 7 replications, pens were allotted to the dietary treatments by BW in a randomized complete block design. Treatment diets were fed for 18 d followed by a common diet for 14 d. Both phases were fed in meal form. Pigs were weighed and feed disappearance was measured on d 0, 7, 14, 18, 25, and 32.

Samples of treatment diets were collected upon manufacturing at the feed mill. Proximate analysis (Ward Laboratories, Inc., Kearney, NE) was conducted on composite samples. Additionally, experimental diet samples were submitted for amino acid analysis (Ajinomoto Heartland, Chicago, IL).

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. A base model was developed and treatments were evaluated as categorical variables with heterogeneity of variance accounted for in the models where appropriate. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ . Feed efficiency was evaluated as G:F and means reported are the reciprocal of G:F values while the  $P$  values reported were those obtained for the analysis of G:F.

Next, PROC GLIMMIX and PROC NLMIXED were used to predict the SID Ile:Lys ratio dose response curves to optimize ADG and ADFI as a function of the dose of dietary Ile:Lys according to procedures of Gonçalves et al. (2016).<sup>5</sup> As feed efficiency resulted in a minor quadratic effect only in Exp. 1, it was not modeled. Models were evaluated separately for individual experiments. Dose response models evaluated were quadratic (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models. Bayesian Information Criterion (BIC) was used to determine best fit, with a lower number indicating an improved fit. As with the base model, heterogeneous variance was accounted for where appropriate.

<sup>5</sup> Gonçalves, M., N. Bello, S. Dritz, M. Tokach, J. DeRouchey, J. Woodworth, and R. Goodband. 2016. An update on modeling dose–response relationships: Accounting for correlated data structure and heterogeneous error variance in linear and nonlinear mixed models. *J. Anim. Sci.* 94(5): 1940-1950.

## Results and Discussion

Amino acid analysis of ingredients (Table 1) resulted in corn generally being slightly higher in AA concentrations as compared to published values with soybean meal being slightly lower. Field pea analysis for Exp. 1 resulted in values much lower than published levels; however, field pea AA levels for Exp. 2 were extremely close to expected values.

Proximate analysis of experimental diets (Tables 4 and 5) generally matched formulated values. Except for the 48 and 52% SID Ile:Lys ratio treatments (Exp. 1), the 40 and 44% SID Ile:Lys ratio treatments (Exp. 2), and the 54 and 58% SID Ile:Lys ratio treatments (Exp. 2), which had equal analyzed Ile content, amino acid analyses of diets were reasonably consistent with diet formulation with Ile generally increasing across the treatments and other AA remaining relatively constant.

### Experiment 1

From d 0 to 12 when experimental diets were fed, ADG and ADFI improved (ADG, linear,  $P < 0.001$ ; ADFI, quadratic,  $P < 0.017$ ) with increasing SID Ile:Lys ratio (Table 6). However, as SID Ile:Lys ratio increased, F/G worsened (quadratic,  $P < 0.041$ ) with the lowest and highest treatments at 40% and 63% SID Ile:Lys ratio having the best feed efficiency creating a quadratic response. During the common phase (d 12 to 28), there were no significant differences in ADG, ADFI or F/G. During the overall period, ADG tended (linear,  $P < 0.082$ ) to improve and ADFI improved (linear,  $P < 0.011$ ) due to increasing SID Ile:Lys ratio in diets from d 0 to 12. Similarly, BW was increased (linear,  $P < 0.006$ ) at the end of Phase 1, but there were no treatment differences detected for final BW.

For ADG (Figures 1), the QP, BLL, and BLQ had competing fits (BIC = 558.3, 556.6, and 557.9, respectively). The QP [ $0.2161539 + 0.0185359 \times (\text{SID Ile:Lys ratio}) - 0.0001435 \times (\text{SID Ile:Lys ratio})^2$ ] reported maximum ADG at 64.7% SID Ile:Lys ratio with 99% of maximum performance captured with 57.0% SID Ile:Lys ratio. The BLL and BLQ reported similar breakpoints of 52.0% SID Ile:Lys ratio (95% CI: [51.96, 52.04%] and [51.97, 52.03%], respectively) with no further improvement in ADG found thereafter. For ADFI, (Figure 2), the BLL and QP resulted in competing fits (BIC = 603.8 and 604.4, respectively). The BLL breakpoint occurred at 50.6% SID Ile:Lys ratio (95% CI: [41.99, 59.15%]). The QP [ $-0.6352513 + 0.0670467 \times (\text{SID Ile:Lys ratio}) - 0.0005963 \times (\text{SID Ile:Lys ratio})^2$ ] reported maximum ADFI at 56.2% SID Ile:Lys ratio with 99% of maximum intake captured at 51.6% SID Ile:Lys ratio.

### Experiment 2

From d 0 to 18 when experimental diets were fed, ADG and ADFI increased (quadratic,  $P < 0.009$ ), but there were no significant differences for F/G as SID Ile:Lys ratio increased (Table 7). During the common period (d 18 to 32), there were no differences for ADG, but ADFI increased (linear,  $P < 0.010$ ) and F/G became poorer (linear,  $P < 0.009$ ) for pigs previously fed diets with increasing SID Ile:Lys ratio. For the overall period, ADG and ADFI increased (quadratic,  $P < 0.034$ ) with increasing SID Ile:Lys ratio with no differences in F/G. Finally, BW was increased (quadratic,  $P < 0.032$ ) at the end of Phase 1 and at the conclusion of the experiment.

For ADG (Figure 3), the BLL and QP were competing best fit models (BIC = 541.8 and 543.3, respectively). The BLL breakpoint occurred at 51.8% SID Ile:Lys ratio (95% CI: [47.65, 55.93%]) with no further improvement thereafter. The QP  $[-0.6856481 + 0.0450816 \times (\text{SID Ile:Lys ratio}) - 0.0003865 \times (\text{SID Ile:Lys ratio})^2]$  reported maximum ADG at 58.3% SID Ile:Lys ratio with 99% of maximum performance captured with 54.3% SID Ile:Lys ratio. For ADFI, (Figure 4), the QP and BLQ resulted in competing fits (BIC = 591.0 and 591.7, respectively). The QP  $[-1.2973325 + 0.0777712 \times (\text{SID Ile:Lys ratio}) - 0.0006795 \times (\text{SID Ile:Lys ratio})^2]$  reported maximum ADFI at 57.2% SID Ile:Lys ratio with 99% of maximum intake captured at 53.5% SID Ile:Lys ratio. The BLQ breakpoint occurred at 52.0% SID Ile:Lys ratio (95% CI: [51.95, 52.05%]).

In conclusion, these experiments demonstrate that the SID Ile requirement for 15 to 25 lb nursery pigs is approximately 52% of Lys for ADG and ADFI using broken line models and can be as high as 64% SID Ile:Lys ratio using quadratic models. A slight quadratic effect was observed in feed efficiency for Exp. 1, however in Exp. 2, there were no appreciable differences detected in F/G. These data validate that the Ile requirement for 15 to 25 lb pigs appears to be similar to NRC (2012) requirement estimates.

**Table 1. Ingredient chemical analysis**

Item, %	Corn <sup>1,2</sup>	Soybean meal <sup>1,2</sup>	Dried whey <sup>1,2</sup>	Field peas, Exp. 1 <sup>3</sup>	Field peas, Exp. 2 <sup>1,3</sup>	Spray dried blood cells <sup>4</sup>
Total Amino acids						
Lys	0.29	2.88	0.79	1.54	1.59	8.88
Ile	0.31	2.09	0.65	0.89	0.91	0.28
Leu	1.10	3.51	1.07	1.52	1.59	12.62
Met	0.18	0.66	0.16	0.19	0.21	1.23
Thr	0.30	1.80	0.68	0.79	0.83	4.29
Trp	0.07	0.65	0.22	0.17	0.21	1.58
Val	0.40	2.12	0.59	0.99	0.99	8.35
His	0.24	1.17	0.18	0.50	0.54	3.17
Phe	0.43	2.35	0.37	1.03	1.07	7.25
Standardized ileal digestible amino acids, % (Calculated)						
Lys	0.21	2.56	0.77	1.40	1.44	8.67
Ile	0.25	1.86	0.62	0.78	0.79	0.25
Leu	0.95	3.09	1.05	1.35	1.41	12.3
Met	0.15	0.59	0.16	0.17	0.19	1.26
Thr	0.23	1.53	0.61	0.69	0.72	4.13
Trp	0.06	0.59	0.21	0.15	0.18	1.48
Val	0.32	1.84	0.56	0.86	0.86	8.14
His	0.20	1.05	0.17	0.46	0.50	6.07
Phe	0.36	2.07	0.34	0.92	0.96	7.08

<sup>1</sup>Analyzed at Ajinomoto Heartland, Inc. (Chicago, IL) for amino acid content.

<sup>2</sup>SID % content calculated using SID coefficients from the NRC (NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC).

<sup>3</sup>Exp. 1 peas use total AA content and SID coefficients from Mathai, J. K. 2015. Effects of Fiber on the Optimum Threonine:Lysine Ratio in 25 to 50 kg Growing Gilts. MS Thesis. University of Illinois at Urbana-Champaign, Urbana. Exp. 2 peas use SID coefficients from Mathai thesis.

<sup>4</sup>Spray dried blood cells use total values and coefficients from Almeida, F., Htoo, J., Thomson, J., and Stein, H. (2013). Comparative amino acid digestibility in US blood products fed to weanling pigs. Animal Feed Science and Technology, 181, 80-86.

**Table 2. Diet composition (Exp. 1, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, %		Common Phase
	40	63	
Ingredient, %			
Corn	57.68	57.59	63.77
Soybean meal (48% CP)	13.25	13.26	32.86
Dried whey	10.00	10.00	--
Field peas	10.00	10.00	--
Spray dried blood cells	1.50	1.50	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate (22% P)	1.80	1.80	1.10
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.63	0.63	0.30
DL-Met	0.33	0.33	0.12
L-Thr	0.32	0.32	0.12
L-Trp	0.10	0.10	--
L-Val	0.24	0.24	--
L-Ile	--	0.29	--
Glutamic acid	1.10	1.00	--
Glycine	1.10	1.00	--
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00

*continued*

**Table 2. Diet composition (Exp. 1, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, %		Common Phase
	40	63	
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.28	1.28	1.22
Ile:Lys	40	63	63
Leu:Lys	107	107	129
Met:Lys	42	42	33
Met and Cys:Lys	59	59	57
Thr:Lys	65	65	63
Trp:Lys	20.3	20.3	18.7
Val:Lys	71	71	69
Total Lys, %	1.38	1.38	1.37
ME, kcal/lb	1,464	1,468	1,484
NE, kcal/lb	1,101	1,105	1,092
SID Lys:ME, g/Mcal	3.96	3.95	3.73
SID Lys:NE, g/Mcal	5.36	5.34	5.16
CP, %	18.2	18.2	21.4
Ca, %	0.82	0.82	0.70
P, %	0.73	0.73	0.64
Available P, %	0.51	0.51	0.41

<sup>1</sup>Treatments 40% and 63% SID Ile:Lys were manufactured and blended at the feed mill to create the intermediate levels of 44, 48, 52, 54, and 58% SID Ile:Lys.

**Table 3. Diet composition (Exp. 2, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, %		Common Phase
	40	63	
Ingredient, %			
Corn	59.04	58.95	63.77
Soybean meal (48% CP)	11.95	11.96	32.86
Dried whey	10.00	10.00	--
Field peas	10.00	10.00	--
Spray dried blood cells	1.50	1.50	--
Limestone	1.00	1.00	0.98
Monocalcium phosphate (22% P)	1.80	1.80	1.10
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.60	0.60	0.30
DL-Met	0.32	0.32	0.12
L-Thr	0.31	0.31	0.12
L-Trp	0.10	0.10	--
L-Val	0.23	0.23	--
L-Ile	--	0.28	--
Glutamic acid	1.10	1.00	--
Glycine	1.10	1.00	--
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Zinc oxide	0.25	0.25	--
Total	100.00	100.00	100.00

*continued*

**Table 3. Diet composition (Exp. 2, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, %		Common Phase
	40	63	
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.24	1.24	1.22
Ile:Lys	40	63	63
Leu:Lys	109	109	129
Met:Lys	42	42	33
Met and Cys:Lys	60	60	57
Thr:Lys	66	66	63
Trp:Lys	21	21	18.7
Val:Lys	71	71	69
Total Lys, %	1.34	1.34	1.37
ME, kcal/lb	1,464	1,468	1,484
NE, kcal/lb	1,104	1,108	1,092
SID Lys:ME, g/Mcal	3.83	3.82	3.73
SID Lys:NE, g/Mcal	5.15	5.14	5.16
CP, %	18.6	18.6	21.4
Ca, %	0.81	0.81	0.70
P, %	0.73	0.73	0.64
Available P, %	0.51	0.51	0.41

<sup>1</sup>Treatments 40% and 63% SID Ile:Lys were manufactured and blended at the feed mill to create the intermediate levels of 44, 48, 52, 54, and 58% SID Ile:Lys.

**Table 4. Chemical analysis of diets (Exp. 1, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, % <sup>2</sup>						
	40	44	48	52	54	58	63
Proximate analysis, % <sup>3</sup>							
DM	88.11	88.82	89.21	88.94	87.85	88.86	89.23
CP	18.0	18.7	18.6	18.7	18.8	18.8	19.0
Crude fiber	2.2	2.0	2.1	2.2	2.3	2.0	2.1
Ether extract	2.6	2.4	2.4	2.6	2.3	2.3	2.1
Ash	4.64	5.52	4.79	5.07	5.3	5.06	5.29
Amino acid analysis, % <sup>4</sup>							
Lys	1.28	1.34	1.40	1.42	1.39	1.45	1.41
Ile	0.59	0.67	0.74	0.74	0.79	0.86	0.93
Leu	1.46	1.50	1.52	1.52	1.53	1.51	1.56
Met	0.46	0.50	0.50	0.53	0.49	0.56	0.55
Met + Cys	0.75	0.79	0.79	0.74	0.76	0.84	0.83
Thr	0.93	0.92	0.89	0.97	0.87	0.91	0.95
Trp	0.24	0.26	0.27	0.28	0.26	0.28	0.29
Val	0.92	0.98	0.97	1.04	1.01	1.04	1.07
His	0.43	0.45	0.45	0.46	0.45	0.45	0.46
Phe	0.79	0.80	0.81	0.81	0.79	0.79	0.81

<sup>1</sup>Treatment diet samples were collected at the feed mill after manufacturing.

<sup>2</sup>Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) diets were blended at the feed mill to create the intermediate treatments.

<sup>3</sup>Composite samples were submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

<sup>4</sup>Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for amino acid analysis.

**Table 5. Chemical analysis of diets (Exp. 2, as-fed basis)<sup>1</sup>**

Item	Formulated SID Ile:Lys ratio, % <sup>2</sup>						
	40	44	48	52	54	58	63
Proximate analysis, % <sup>3</sup>							
DM	90.29	90.41	90.07	90.37	90.36	90.30	89.97
CP	18.1	18.4	18.6	18.2	18.3	18.7	18.7
Crude fiber	1.9	1.8	2.4	1.7	1.9	1.9	2.3
Ether extract	2.6	2.7	2.4	2.5	2.6	2.7	2.6
Ash	5.25	5.27	5.12	5.24	5.12	5.40	5.18
Amino acid analysis, % <sup>4</sup>							
Lys	1.33	1.34	1.34	1.36	1.36	1.35	1.33
Ile	0.60	0.60	0.65	0.69	0.75	0.75	0.82
Leu	1.47	1.43	1.45	1.47	1.48	1.47	1.48
Met	0.50	0.54	0.51	0.50	0.52	0.50	0.54
Met + Cys	0.75	0.77	0.73	0.75	0.76	0.77	0.82
Thr	0.86	0.84	0.92	0.87	0.90	0.91	0.98
Trp	0.27	0.27	0.27	0.25	0.27	0.26	0.27
Val	0.97	0.96	0.96	0.95	0.99	1.02	0.99
His	0.46	0.43	0.45	0.43	0.45	0.44	0.45
Phe	0.79	0.77	0.78	0.78	0.78	0.78	0.78

<sup>1</sup>Treatment diet samples were collected at the feed mill after manufacturing.

<sup>2</sup>Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) diets were blended at the feed mill to create the intermediate treatments.

<sup>3</sup>Composite samples were submitted to Ward Laboratories (Kearney, NE) for proximate analysis.

<sup>4</sup>Composite samples were submitted to Ajinomoto Heartland Inc. (Chicago, IL) for amino acid analysis.

**Table 6. Effects of increasing SID Ile:Lys ratio on nursery pig growth performance, Exp. 1<sup>1,2</sup>**

Item	Formulated SID Ile:Lys ratio, % <sup>3</sup>							SEM	Probability <sup>4</sup> , <i>P</i> <	
	40	44	48	52	54	58	63		Linear	Quadratic
Phase 1 (d 0 to 12)										
ADG, lb	0.73	0.76	0.76	0.86	0.76	0.79	0.83	0.025	0.001	0.446
ADFI, lb	1.09	1.16	1.20	1.33	1.15	1.27	1.22	0.037	0.002	0.017
F/G <sup>5</sup>	1.50	1.53	1.60	1.55	1.52	1.61	1.49	0.037	0.900	0.041
Phase 2 (d 12 to 26)										
ADG, lb	1.22	1.22	1.23	1.22	1.26	1.27	1.20	0.032	0.779	0.325
ADFI, lb	1.88	1.84	1.88	1.89	1.91	1.96	1.88	0.043	0.190	0.588
F/G	1.54	1.51	1.53	1.56	1.51	1.55	1.57	0.024	0.159	0.366
Overall (d 0 to 26)										
ADG, lb	0.99	1.01	1.01	1.05	1.03	1.05	1.03	0.024	0.082	0.270
ADFI, lb	1.51	1.52	1.57	1.63	1.56	1.64	1.58	0.034	0.011	0.106
F/G	1.52	1.51	1.55	1.55	1.52	1.57	1.54	0.022	0.317	0.577
BW, lb										
d 0	14.9	14.9	14.9	14.9	14.9	14.9	14.9	0.18	0.995	0.993
d 12	23.6	23.9	23.9	25.1	23.9	24.3	24.8	0.41	0.006	0.536
d 26	40.7	41.1	41.1	42.2	41.6	41.1	41.6	0.73	0.105	0.304

<sup>1</sup> A total of 280 nursery pigs (PIC 327 × 1050, initially 14.9 lb BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets.

<sup>2</sup> Experimental diets were fed from d 0 to 12 and a common Phase 3 diet was fed from d 12 to 26.

<sup>3</sup> Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) complete diets were blended upon manufacturing at the feed mill to create the 44, 48, 52, 54, and 58% SID Ile:Lys dietary treatments.

<sup>4</sup> *P* values reported for F/G were analyzed for G:F means.

<sup>5</sup> Means reported for F/G are the reciprocal of G:F.

**Table 7. Effects of increasing SID Ile:Lys ratio on nursery pig growth performance, Exp. 2<sup>1,2</sup>**

Item	Formulated SID Ile:Lys ratio, % <sup>3</sup>							SEM	Probability <sup>4</sup> , <i>P</i> <	
	40	44	48	52	54	58	63		Linear	Quadratic
Phase 1 (d 0 to 18)										
ADG, lb	0.50	0.54	0.59	0.67	0.58	0.63	0.62	0.024	0.001	0.009
ADFI, lb	0.73	0.82	0.87	1.00	0.87	0.93	0.90	0.036	0.001	0.002
F/G <sup>5</sup>	1.46	1.50	1.48	1.48	1.50	1.47	1.46	0.032	0.935	0.228
Phase 2 (d 18 to 32)										
ADG, lb	1.24	1.30	1.29	1.29	1.27	1.31	1.29	0.035	0.246	0.378
ADFI, lb	1.88	2.00	1.97	2.07	1.99	2.05	2.04	0.057	0.010	0.154
F/G	1.51	1.54	1.54	1.60	1.56	1.57	1.58	0.023	0.009	0.298
Overall (d 0 to 32)										
ADG, lb	0.83	0.88	0.89	0.95	0.88	0.93	0.91	0.025	0.001	0.034
ADFI, lb	1.23	1.33	1.35	1.47	1.36	1.42	1.40	0.041	0.001	0.010
F/G	1.49	1.52	1.51	1.55	1.54	1.53	1.54	0.021	0.107	0.209
BW, lb										
d 0	13.3	13.3	13.3	13.3	13.3	13.3	13.3	0.59	0.824	0.920
d 18	22.3	23.1	23.9	25.4	23.8	24.7	24.5	0.91	0.001	0.010
d 32	39.7	41.3	41.9	43.5	41.7	43.0	42.5	1.30	0.001	0.032

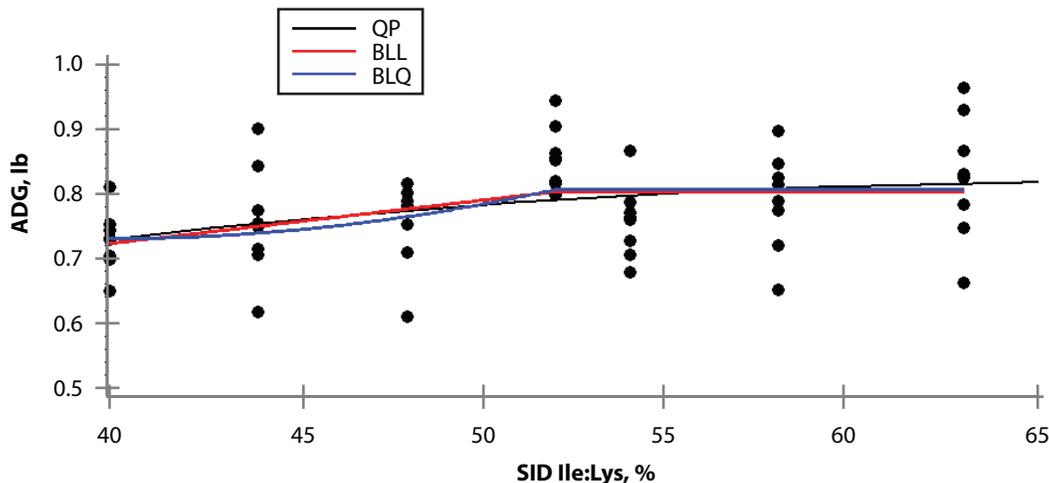
<sup>1</sup> A total of 280 nursery pigs (DNA Genetics Line 600 × Line 241, initially 13.3 lb BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets.

<sup>2</sup> Experimental diets were fed from d 0 to 18 and a common Phase 3 diet was fed from d 18 to 32.

<sup>3</sup> Low (40% SID Ile:Lys) and high (63% SID Ile:Lys) complete diets were blended upon manufacturing at the feed mill to create the 44, 48, 52, 54, and 58% SID Ile:Lys dietary treatments.

<sup>4</sup> *P* values reported for F/G were analyzed for G:F means.

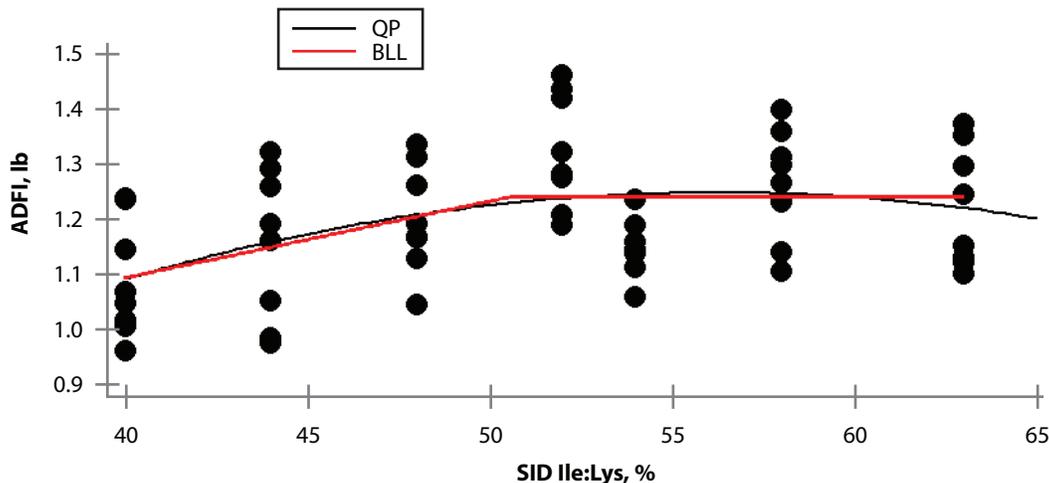
<sup>5</sup> Means reported for F/G are the reciprocal of G:F.



**Figure 1. Estimation of the SID Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 1.**

**ADG QP** BIC= 558.3  
 Max 64.7% SID Ile:Lys;  
 99% of Max 57.0 % SID Ile:Lys  
 $[0.2161539 + 0.0185359 \times (\text{SID Ile:Lys}) - 0.0001435 \times (\text{SID Ile:Lys})^2]$   
**BLL** BIC= 556.6  
 Breakpoint 52.0% SID Ile:Lys  
 95% CI:[51.96, 52.04]  
**BLQ** BIC= 557.9  
 Breakpoint 52.0% SID Ile:Lys  
 95% CI:[51.97, 52.03]

A total of 280 nursery pigs (PIC 327 × 1050, initially 14.9 lb BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 12 and a common Phase 3 diet was fed from d 12 to 26. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting model.



**Figure 2. Estimation of the SID Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 1.**

**ADFI QP** BIC= 604.4  
 Max 56.2% SID Ile:Lys  
 99% of Max 51.6% SID Ile:Lys  
 $[-0.6352513 + 0.0670467 \times (\text{SID Ile:Lys}) - 0.0005963 \times (\text{SID Ile:Lys})^2]$   
**BLL** BIC= 603.8  
 Breakpoint 50.6% SID Ile:Lys  
 95% CI:[41.99, 59.15]

A total of 280 nursery pigs (PIC 327 × 1050, initially 14.9 lb BW) were used in a 26-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 12 and a common Phase 3 diet was fed from d 12 to 26. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting model.

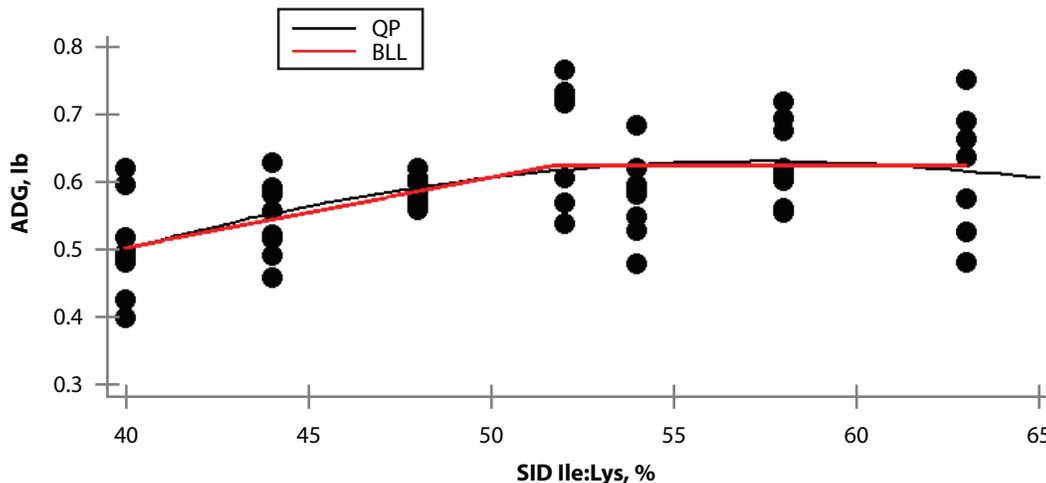


Figure 3. Estimation of the SID Ile:Lys ratio requirement to maximize ADG for nursery pigs, Exp. 2.

**ADG QP** BIC= 543.3  
 Max 58.3% SID Ile:Lys  
 99% of Max 54.3% SID Ile:Lys  
 $[-0.6856481 + 0.0450816 \times (\text{SID Ile:Lys}) - 0.0003865 \times (\text{SID Ile:Lys})^2]$   
**BLL** BIC= 541.8  
 Breakpoint 51.8% SID Ile:Lys  
 95% CI:[47.65, 55.93]

A total of 280 nursery pigs (DNA Genetics Line 600 × Line 241, initially 13.3 lb BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 18 and a common Phase 3 diet was fed from d 18 to 32. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADG. Bayesian Information Criterion (BIC) was used to determine the best fitting model.

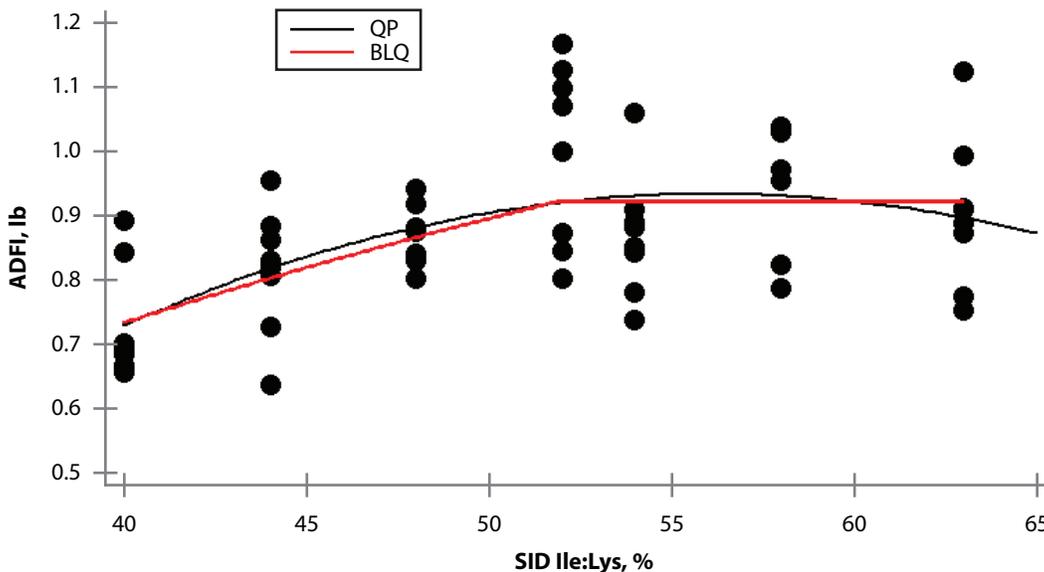


Figure 4. Estimation of the SID Ile:Lys ratio requirement to maximize ADFI for nursery pigs, Exp. 2.

ADFI QP BIC= 591.0  
 Max 57.2% SID Ile:Lys  
 99% of Max 53.5% SID Ile:Lys  
 $[-1.2973325 + 0.0777712 \times (\text{SID Ile:Lys}) - 0.0006795 \times (\text{SID Ile:Lys})^2]$   
 BLQ BIC= 591.7  
 Breakpoint 52.0% SID Ile:Lys  
 95% CI:[51.95, 52.05]

A total of 280 nursery pigs (DNA Genetics Line 600 × Line 241, initially 13.3 lb BW) were used in a 32-d growth trial with 5 pigs per pen and 8 pens per treatment. Pigs were weaned at approximately 20 d of age. One replication was fed a common starter diet for 3 days due to increased weaning BW, and the other seven replications were fed a common starter diet for 6 d post-weaning, then placed on experimental diets. Experimental diets were fed from d 0 to 18 and a common Phase 3 diet was fed from d 18 to 32. Quadratic polynomial (QP), broken-line linear (BLL), and broken-line quadratic (BLQ) models were fit for the experimental period to estimate SID Ile:Lys ratio to maximize ADFI. Bayesian Information Criterion (BIC) was used to determine the best fitting model.

## Effects of AminoGut and Diet Formulation Approach on Growth Performance and Economic Return in Nursery Pigs<sup>1,2</sup>

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### Summary

Diets containing animal protein sources have higher levels of glutamine than diets based on plant protein sources. Therefore, the objective of this study was to determine the effects of AminoGut (Ajinomoto Heartland, Inc., Chicago, IL) and protein source (animal vs. plant proteins) on growth performance and economic return in nursery pigs from 12 to 60 lb. AminoGut is a product that contains both glutamine and glutamate. A total of 1,134 pigs (337 × 1050; PIC, Hendersonville, TN, initially 11.6 ± 0.18 lb BW) were used in a 52-d trial. At the beginning of the experiment, pigs were weighed in pens, and pens were ranked by average BW and randomly assigned dietary treatments in a randomized complete block design based on BW. The treatment structure was a 2 × 3 factorial with 2 protein sources (animal vs. plant) and 3 AminoGut durations (0, 10, and 24 d). The experiment was divided into Phases 1 (d 0 to 10), 2 (d 10 to 24), and 3 (d 24 to 52). Pigs were fed a common diet during Phase 3. AminoGut was added at 0.8 and 0.6% in Phases 1 and 2, respectively. From d 0 to 10, pigs fed animal protein-based diet had marginally ( $P = 0.074$ ) greater ADG and improved F/G ( $P = 0.035$ ) compared to pigs fed plant-based diet. No evidence for differences was observed in pigs fed AminoGut in this phase ( $P > 0.188$ ). From d 10 to 24, pigs fed AminoGut had improved ADG (linear,  $P < 0.022$ ) and F/G (linear,  $P = 0.004$ ). No evidence for differences was observed between protein sources in this phase. From d 24 to 52, pigs that had been previously fed AminoGut for 10 d had marginally improved F/G (quadratic,  $P = 0.057$ ) compared to pigs not previously fed AminoGut or previously fed AminoGut for 24 d. No evidence for differences was observed between protein sources in this common phase. For the combined performance from Phases 1 and 2 (d 0 to 24), pigs fed AminoGut had improved ADG (linear,  $P < 0.021$ ), F/G (linear,  $P = 0.004$ ), and BW (quadratic,  $P = 0.028$ ) compared to pigs not fed AminoGut. No evidence for differences was observed between pigs fed different protein sources. For the overall performance

<sup>1</sup> The authors thank Ajinomoto Heartland Inc., Chicago, IL, for providing feed-grade amino acids and for partial financial support.

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<sup>4</sup> Ajinomoto Heartland Inc., Chicago, IL.

(d 0 to 52), no statistical evidence for differences between pigs fed protein source or different AminoGut duration was observed. In conclusion, feeding AminoGut for 10 d post-weaning marginally improved growth performance until d 24 but there was no carry over effect when a common diet was fed from d 24 to 52. Further research should evaluate the supplementation of glutamine and glutamate throughout the nursery period and at greater inclusion levels.

Key words: glutamate, glutamine, growth, nursery pig, protein source

## Introduction

Glutamine is considered a non-essential amino acid for young pigs and is important for optimum health and function of the enterocytes in the small intestine. Glutamate is an important compound in cellular metabolism and is an energy source for the enterocytes in the intestine. Dietary glutamate may be limiting in newly-weaned pigs due to rapid turnover and replacement of mucosal cells. These are the most abundant amino acids in milk. AminoGut (Ajinomoto Heartland, Inc., Chicago, IL) is a product that contains both glutamine and glutamate. There may be a benefit of glutamine and glutamate supplementation in newly-weaned pigs on jejunal atrophy and growth performance (Wu et al., 1996;<sup>5</sup> Rezaei et al., 2012;<sup>6</sup> Cabrera et al., 2013<sup>7</sup>). Additionally, dietary animal protein sources have greater glutamine content compared to plant protein sources. Therefore, the objective of this study was to determine the effects of AminoGut and protein source on growth performance and economic return in nursery pigs from 12 to 60 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at a commercial research nursery barn in southwestern Minnesota. The facility was totally enclosed, environmentally controlled, and mechanically ventilated. Pens had completely slatted flooring and deep pits for manure storage. Each pen (12 × 7.5 ft) was equipped with a 6-hole stainless steel, dry self-feeder (SDI Industries, Alexandria, SD) and a pan waterer for ad libitum access to feed and water.

A total of 1,134 pigs (PIC 337 × 1050; initial BW of 11.6 ± 0.18 lb) were used in a 52-d growth trial. There were 7 pens per treatment and 27 pigs per pen. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts for individual pens. This system is capable of feeding each individual pen any of the individual diets as well as a blend of 2 diets.

<sup>5</sup> Wu, G., M. Sa, and K. Da. 1996. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *J. Nutr.* 126:2578–2584.

<sup>6</sup> Rezaei, R., D. A. Knabe, C. D. Tekwe, S. Dahanayaka, M. D. Ficken, S. E. Fielder, S. J. Eide, S. L. Lovering, and G. Wu. 2012. Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. *Amino Acids* 44:911–923.

<sup>7</sup> Cabrera, R. A., Usry, J. L., Arrellano, C., Nogueira, E. T., Kutschenko, M., Moeser, A. J., and Odle, J. 2013. Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (AminoGut) on pre- and post-weaning growth performance and intestinal health of piglets. *J. Anim. Sci. Biot.*, 4:1-13.

Pens were randomly assigned within weight blocks to dietary treatments. Dietary treatments (Table 1) were arranged as a  $2 \times 3$  factorial with 2 diet formulation approaches (animal or plant protein) and 3 feeding durations of AminoGut (0, 10, or 24 d). The AminoGut inclusion rate was 0.8% from d 0 to 10 and 0.6% from d 10 to 24. From d 24 to 52, pigs were fed a common diet. Pig BW and feed disappearance were measured on d 0, 10, 24, and 52 to calculate ADG, ADFI, and F/G.

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the experiment and stored at  $-4^{\circ}\text{F}$  until analyzed. Total amino acids and CP analyses were conducted on composite samples from each dietary treatment (Ajinomoto Heartland, Inc). Diet samples were also submitted for analysis of DM, crude fiber, ADF, NDF, ash, ether extract, Ca, and P (Ward Laboratories, Inc Kearney, NE).

For the economic evaluation, total feed cost per pig, cost per lb of gain, revenue, and income over feed cost (IOFC) were calculated on a pen basis. The total feed cost per pig was calculated by multiplying the ADFI by diet cost and the number of days it was fed. Cost per lb of gain was calculated by dividing the total feed cost per pig by overall pounds gained. Revenue per pig was calculated by multiplying the ADG times the total days in the trial times an assumed live price of \$54.00 per cwt. To calculate IOFC, total feed cost was subtracted from pig revenue. For all economic evaluations, price of ingredients were: corn at \$3.60/bu (\$129/ton), DDGS at \$180/ton, soybean meal at \$354/ton, L-tryptophan at \$6.00/lb, L-valine at \$7.50/lb, and AminoGut at \$2.34/lb.

Responses measured at the pen level were analyzed using a linear mixed model. The model included the fixed effect of treatment and initial pig BW as a random effect. Pen was the experimental unit. Linear and quadratic contrasts were built to evaluate the dose response to feeding AminoGut for different durations. Statistical models were fitted using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC). Results were considered significant at  $P \leq 0.05$  and marginally significant at  $P \leq 0.10$ .

## Results and Discussion

The analyzed total amino acids, DM, CP, crude fiber, Ca, P, fat and ash contents of experimental diets (Table 2) were reasonably consistent with formulated estimates.

There were no interactions observed between protein source and AminoGut duration with the exception of BW at d 10, which was marginally heavier ( $P = 0.093$ ) for pigs fed animal protein-based diet with 24 d of AminoGut duration (Table 3) compared with pigs fed vegetable-based diet with 24 d of AminoGut duration.

From d 0 to 10, pigs fed the animal protein-based diet had marginally ( $P = 0.074$ ) greater ADG and improved F/G ( $P = 0.035$ ) compared to pigs fed the plant-based diet. No evidence for differences was observed among pigs fed AminoGut in this phase ( $P > 0.188$ ). From d 10 to 24, pigs fed AminoGut had improved ADG (linear,  $P < 0.022$ ) and F/G (linear,  $P = 0.004$ ). No evidence for differences was observed between protein sources in this phase. From d 24 to 52, pigs that had been previously fed AminoGut for 10 d had marginally improved F/G (quadratic,  $P = 0.057$ ) compared to pigs not previ-

ously fed AminoGut or previously fed AminoGut for 24 d. No evidence for differences was observed between protein sources in this common phase.

For the combined performance from Phases I and II (d 0 to 24), pigs fed AminoGut had improved ADG (linear,  $P < 0.021$ ), F/G (linear,  $P = 0.004$ ), and BW (quadratic,  $P = 0.028$ ) compared to pigs not fed AminoGut. No evidence for differences was observed between pigs fed different protein sources. For the overall performance (d 0 to 52), no statistical evidence for differences between pigs fed either animal- or plant-based protein source or different AminoGut durations was observed

Feed cost per pig was greater ( $P < 0.001$ ) in pigs fed animal protein source compared to plant protein source. Additionally, feed cost per pig increased (linear,  $P = 0.002$ ) with increasing AminoGut duration. Feed cost per lb of gain increased (quadratic,  $P = 0.020$ ) with increasing duration of AminoGut supplementation and also increased ( $P < 0.001$ ) in pigs fed animal protein compared to plant protein. No evidence for differences was observed in total revenue per pig or IOFC.

In conclusion, feeding AminoGut for 10 d post-weaning marginally improved growth performance until d 24 but there was no carry over effect when a common diet was fed from d 24 to 52. Further research should evaluate the supplementation of glutamine and glutamate throughout the nursery period and at greater inclusion levels.

**Table 1. Diet composition (as-fed)<sup>1</sup>**

Protein source:	Phase 1		Phase 2		Common
	Plant	Animal	Plant	Animal	
Ingredient					
Corn	37.92	39.00	46.12	47.15	54.70
Soybean meal (46% CP)	18.05	17.95	25.09	25.06	28.82
Bovine blood plasma	---	4.00	---	---	---
DDGS <sup>2</sup>	5.00	5.00	7.50	7.50	10.00
Fish meal	---	2.50	---	5.00	---
HP 300 (Hamlet Protein)	6.50	---	5.00	---	---
Spray-dried whey	25.00	25.00	9.00	9.00	---
Corn oil	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.95	0.50	1.03	0.45	1.00
Limestone	0.95	0.90	1.03	0.75	1.10
Salt	0.30	0.30	0.35	0.35	0.35
L-Lys HCL	0.545	0.350	0.500	0.425	0.450
DL-Met	---	---	0.190	0.150	0.130
Methionine hydroxy analog	0.271	0.177	---	---	---
L-Thr	0.190	0.110	0.180	0.160	0.150
L-Trp	0.036	0.013	0.021	0.029	0.021
L-Val	0.110	0.025	0.250	0.035	---
Choline chloride, 60%	0.035	0.035	---	---	---
Zinc oxide	0.400	0.400	0.250	0.250	
Vitamin E, 20,000 IU	0.050	0.050	---	---	---
AminoGut <sup>3</sup>	---	---	---	---	---
Trace mineral premix <sup>4</sup>	0.100	0.100	0.100	0.100	0.100
Vitamin premix <sup>5</sup>	0.125	0.125	0.125	0.125	0.125
Aureo-90 <sup>6</sup>	0.445	0.445	0.445	0.445	---
TBCC <sup>7</sup>	---	---	---	---	0.025
Phytase <sup>8</sup>	0.025	0.025	0.025	0.025	0.025
Total	100	100	100	100	100

*continued*

**Table 1. Diet composition (as-fed)<sup>1</sup>**

Protein source:	Phase 1		Phase 2		Common
	Plant	Animal	Plant	Animal	
Standardized ileal digestible (SID) AA, %					
Lys	1.40	1.40	1.34	1.34	1.27
Ile:Lys	55	55	57	57	59
Leu:Lys	110	118	119	120	130
Met:Lys	36	32	36	36	34
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	62	62	62	62	62
Trp:Lys	18.5	18.5	18.5	18.5	18.5
Val:Lys	66	66	66	66	66
Total Lys, %	1.54	1.56	1.50	1.51	1.43
ME, kcal/lb	1,568	1,576	1,568	1,576	1,559
NE NRC, kcal/lb	1,182	1,188	1,167	1,176	1,157
SID Lys:ME, g/Mcal	4.05	4.04	3.88	3.85	3.70
CP, %	20.5	21.3	21.7	22.0	21.6
Ca, %	0.75	0.75	0.68	0.68	0.68
P, %	0.69	0.66	0.63	0.62	0.62
Available P, %	0.59	0.59	0.36	0.36	0.33
Stand. Dig. P with phytase, %	0.60	0.60	0.49	0.49	0.46
Ca:P	1.09	1.12	0.40	0.39	0.38

<sup>1</sup> Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for CP and total amino acid concentrations and NRC (2012) SID digestibility values were used in the diet formulation.

<sup>2</sup> Dried distillers grains with solubles.

<sup>3</sup> AminoGut (Ajinomoto Heartland, Inc., Chicago, IL) is a product that contains both glutamine and glutamate. AminoGut was included at 0.80% and 0.60% in Phases 1 and 2, respectively, at the expense of corn.

<sup>4</sup> Provided per lb of diet: 33 ppm Mn from manganese oxide, 110 ppm Fe from iron sulfate, 110 ppm Zn from zinc oxide, 16.5 ppm Cu from copper sulfate, 0.33 ppm I from ethylenediamin dihydroiodide, and 0.30 ppm Se from sodium selenite.

<sup>5</sup> Provided per lb of diet: 4,000 IU vitamin A; 625 IU vitamin D<sub>3</sub>; 20 IU vitamin E; 2.0 mg vitamin K; 12.5 mg pantothenic acid; 22.5 mg niacin; and 3.5 mg riboflavin and 15 µg vitamin B12.

<sup>6</sup> Aureo-90 (Zoetis, New York City, NY) provided 800 g/ton of chlortetracycline.

<sup>7</sup> TBCC (Intellibond C; Micronutrients Inc., Indianapolis, IN) provided 145 ppm of tribasic copper chloride.

<sup>8</sup> OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 568 phytase units (FTU) per lb of diet.

**Table 2. Chemical analysis of diets (as-fed)<sup>1,2</sup>**

Protein source:	Phase 1				Phase 2				
	Plant		Animal		Plant		Animal		
	AminoGut:	0.00%	0.80%	0.00%	0.80%	0.00%	0.60%	0.00%	0.60%
Proximate analysis, %									
DM	92.64 (87.65)	93.18 (86.94)	92.63 (88.02)	92.87 (87.31)	91.28 (86.45)	91.92 (85.92)	91.63 (86.98)	92.06 (86.45)	
CP	19.3 (20.5)	20.1 (21.3)	20.3 (21.3)	21.2 (22.1)	20.7 (21.7)	21.9 (22.2)	21.6 (22)	23.2 (22.5)	
CF	1.9 (2.1)	1.5 (2.1)	1.5 (1.9)	1.6 (1.9)	1.8 (2.7)	1.8 (2.7)	1.9 (2.6)	2 (2.6)	
ADF	2.2 (2.6)	2.9 (2.6)	2.2 (2.7)	2.5 (2.7)	3.1 (3.6)	3.3 (3.5)	3.1 (3.6)	3.6 (3.6)	
NDF	7.5 (6.5)	7.5 (6.4)	7.9 (6.6)	7.5 (6.5)	9.4 (8.5)	10.7 (8.5)	9.9 (8.6)	8.7 (8.6)	
Ca	1.01 (0.75)	0.86 (0.75)	1.04 (0.75)	0.94 (0.75)	0.90 (0.68)	0.84 (0.68)	0.89 (0.68)	0.94 (0.68)	
P	0.63 (0.69)	0.62 (0.68)	0.62 (0.66)	0.59 (0.66)	0.66 (0.63)	0.7 (0.63)	0.68 (0.62)	0.69 (0.62)	
Fat	5.7 (5.3)	5.9 (5.3)	6.1 (5.5)	6.1 (5.5)	5.4 (5.7)	5.5 (5.7)	6 (6.1)	6 (6.1)	
Ash	6.68 (4.27)	6.2 (4.26)	6.22 (4.59)	6.06 (4.58)	5.63 (3.63)	5.83 (3.62)	5.72 (4.1)	6.12 (4.09)	
Amino acids, %									
Lysine	1.40 (1.54)	1.43 (1.54)	1.53 (1.56)	1.58 (1.56)	1.32 (1.50)	1.37 (1.50)	1.46 (1.51)	1.47 (1.51)	
Isoleucine	0.82 (0.86)	0.84 (0.86)	0.87 (0.88)	0.86 (0.88)	0.83 (0.88)	0.80 (0.88)	0.81 (0.89)	0.81 (0.89)	
Leucine	1.71 (1.72)	1.73 (1.72)	1.79 (1.88)	1.77 (1.87)	1.60 (1.82)	1.56 (1.81)	1.70 (1.85)	1.70 (1.84)	
Methionine <sup>3</sup>	0.28 (0.52)	0.27 (0.52)	0.31 (0.53)	0.30 (0.53)	0.44 (0.53)	0.48 (0.53)	0.49 (0.48)	0.50 (0.48)	
Met and Cys <sup>3</sup>	0.57 (0.86)	0.57 (0.86)	0.66 (0.86)	0.66 (0.86)	0.75 (0.87)	0.81 (0.87)	0.83 (0.89)	0.85 (0.89)	
Threonine	0.96 (1.00)	0.93 (0.99)	0.95 (1.02)	0.94 (1.02)	0.95 (0.97)	0.91 (0.96)	0.99 (0.97)	0.97 (0.97)	
Tryptophan	0.27 (0.29)	0.28 (0.29)	0.28 (0.29)	0.27 (0.29)	0.26 (0.28)	0.26 (0.28)	0.28 (0.28)	0.27 (0.28)	
Valine	0.95 (1.05)	0.96 (1.04)	1.00 (1.08)	0.99 (1.08)	0.97 (1.03)	0.94 (1.03)	0.97 (1.04)	0.99 (1.04)	
Histidine	0.50 (0.50)	0.50 (0.50)	0.53 (0.55)	0.51 (0.55)	0.45 (0.56)	0.44 (0.56)	0.48 (0.56)	0.48 (0.56)	
Phenylalanine	0.95 (0.92)	0.95 (0.92)	1.00 (0.97)	0.99 (0.97)	0.86 (1.03)	0.85 (1.02)	0.90 (1.01)	0.88 (1.01)	

<sup>1</sup> Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d prior to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland, Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, crude fiber, Ca, P, ash, and crude fat.

<sup>2</sup> Values in parentheses indicate those calculated from diet formulation and are based on values from NRC (2012).

<sup>3</sup> Difference between analyzed and formulated value is due to the utilization of methionine hydroxyl analog in diet formulation not being recovered during chemical analysis.

**Table 3. Effects of AminoGut and diet formulation approach (plant or animal protein) on growth performance and economics of nursery pigs<sup>1,2</sup>**

Protein source: AminoGut duration, d:	Protein source:						SEM	Probability, <i>P</i> <				
	Vegetable			Animal				AminoGut duration, d		Protein source	AminoGut × Protein source	
	0	10	24	0	10	24		Linear	Quadratic		Linear	Quadratic
d 0 to 10												
ADG, lb	0.19	0.17	0.18	0.19	0.19	0.21	0.012	0.392	0.188	0.074	0.204	0.855
ADFI, lb	0.39	0.39	0.38	0.38	0.38	0.39	0.008	0.853	0.804	0.379	0.237	0.648
F/G	2.13	2.37	2.16	2.06	2.02	1.87	0.139	0.557	0.232	0.035	0.437	0.439
d 10 to 24												
ADG, lb	0.73	0.77	0.80	0.75	0.79	0.79	0.022	0.022	0.553	0.503	0.331	0.653
ADFI, lb	1.00	1.00	1.04	1.03	1.04	1.03	0.019	0.197	0.844	0.166	0.294	0.364
F/G	1.37	1.31	1.30	1.37	1.32	1.32	0.020	0.004	0.150	0.526	0.713	0.841
d 0 to 24												
ADG, lb	0.50	0.52	0.54	0.51	0.54	0.54	0.014	0.021	0.913	0.236	0.789	0.727
ADFI, lb	0.74	0.74	0.76	0.75	0.76	0.76	0.012	0.219	0.841	0.290	0.626	0.549
F/G	1.49	1.44	1.42	1.47	1.42	1.40	0.024	0.004	0.625	0.360	0.963	0.941
d 24 to 52												
ADG, lb	1.19	1.23	1.21	1.22	1.24	1.22	0.017	0.552	0.101	0.188	0.456	0.558
ADFI, lb	1.80	1.85	1.84	1.85	1.86	1.87	0.022	0.147	0.511	0.125	0.457	0.373
F/G	1.51	1.50	1.53	1.52	1.50	1.53	0.011	0.168	0.057	0.835	0.888	0.651

*continued*

**Table 3. Effects of AminoGut and diet formulation approach (plant or animal protein) on growth performance and economics of nursery pigs<sup>1,2</sup>**

Protein source: AminoGut duration, d:	Vegetable						Animal						Probability, <i>P</i> <				
	0			10			24			SEM			AminoGut duration, d		Protein source	AminoGut × Protein source	
	0	10	24	0	10	24	SEM	Linear	Quadratic	Linear	Quadratic						
<b>d 0 to 52</b>																	
ADG, lb	0.87	0.90	0.90	0.89	0.91	0.91	0.015	0.154	0.247	0.194	0.578	0.853					
ADFI, lb	1.30	1.34	1.34	1.34	1.35	1.35	0.015	0.141	0.547	0.147	0.474	0.632					
F/G	1.51	1.49	1.49	1.50	1.48	1.49	0.013	0.469	0.181	0.703	0.932	0.755					
<b>BW, lb</b>																	
d 0	11.6	11.6	11.6	11.6	11.5	11.6	0.18	0.725	0.636	0.566	0.414	0.314					
d 10	13.5	13.3	13.4	13.5	13.5	13.8	0.20	0.516	0.211	0.060	0.093	0.864					
d 24	23.8	24.1	24.8	24.2	24.7	24.9	0.42	0.028	0.848	0.191	0.739	0.580					
d 52	57.2	58.5	58.8	58.5	59.3	59.1	0.84	0.149	0.430	0.185	0.552	0.987					
<b>Economics, \$</b>																	
Feed cost/pig	10.47	10.73	11.00	11.18	11.30	11.56	0.130	0.002	0.770	0.001	0.536	0.771					
Feed cost/lb gain <sup>3</sup>	0.232	0.230	0.236	0.241	0.239	0.246	0.002	0.056	0.020	0.001	0.880	0.918					
Total revenue/pig <sup>4</sup>	24.35	25.23	25.16	25.05	25.58	25.31	0.41	0.154	0.247	0.194	0.578	0.853					
IOFC <sup>5</sup>	13.88	14.50	14.16	13.87	14.27	13.86	0.308	0.662	0.102	0.475	0.637	0.904					

<sup>1</sup> A total of 1,134 pigs (PIC 337 × 1050, initially 11.6 lb BW) were used in a 52-d growth trial with 27 pigs per pen and 7 pens per treatment.

<sup>2</sup> Corn was valued at \$3.60/bu (\$129/ton), DDGS at \$180/ton, soybean meal at \$354/ton, and AminoGut at \$2.34/lb.

<sup>3</sup> Feed cost/lb gain = total feed cost divided by total gain per pig. Cost per ton used is not considering processing costs.

<sup>4</sup> One lb of live gain was considered to be worth \$0.68. Total revenue/pig = total gain/pig × \$0.54.

<sup>5</sup> Income over feed cost = total revenue/pig – feed cost/pig.

**Table 4. Main effects of AminoGut duration and diet formulation approach (plant vs. animal proteins) on growth performance and economics in nursery pigs<sup>1,2</sup>**

Item	Protein source			Probability, <i>P</i> <	AminoGut duration, d				Probability, <i>P</i> <
	Plant	Animal	SEM		0	10	24	SEM	
d 0 to 10									
ADG, lb	0.18 <sup>x</sup>	0.20 <sup>y</sup>	0.01	0.074	0.19	0.18	0.20	0.01	0.291
ADFI, lb	0.39	0.38	0.01	0.379	0.38	0.38	0.38	0.01	0.953
F/G	2.22 <sup>a</sup>	1.98 <sup>b</sup>	0.09	0.032	2.09	2.19	2.02	0.10	0.400
d 10 to 24									
ADG, lb	0.76	0.78	0.01	0.497	0.74 <sup>x</sup>	0.78 <sup>y</sup>	0.79 <sup>y</sup>	0.02	0.056
ADFI, lb	1.01	1.03	0.01	0.166	1.01	1.02	1.04	0.01	0.421
F/G	1.33	1.34	0.01	0.515	1.37 <sup>a</sup>	1.31 <sup>b</sup>	1.31 <sup>b</sup>	0.01	0.005
d 0 to 24									
ADG, lb	0.52	0.53	0.01	0.225	0.51 <sup>x</sup>	0.53 <sup>xy</sup>	0.54 <sup>y</sup>	0.01	0.059
ADFI, lb	0.75	0.76	0.01	0.280	0.75	0.75	0.76	0.01	0.438
F/G	1.45	1.43	0.01	0.347	1.48 <sup>a</sup>	1.43 <sup>b</sup>	1.41 <sup>b</sup>	0.02	0.011
d 24 to 52									
ADG, lb	1.21	1.23	0.01	0.180	1.21	1.23	1.21	0.01	0.204
ADFI, lb	1.83	1.86	0.01	0.122	1.82	1.85	1.86	0.02	0.273
F/G	1.51	1.52	0.01	0.830	1.51 <sup>xy</sup>	1.50 <sup>x</sup>	1.53 <sup>y</sup>	0.01	0.057
d 0 to 52									
ADG, lb	0.89	0.90	0.01	0.182	0.88	0.90	0.90	0.01	0.172
ADFI, lb	1.33	1.35	0.01	0.138	1.32	1.34	1.35	0.01	0.265
F/G	1.50	1.49	0.01	0.696	1.50	1.48	1.49	0.01	0.295
BW, lb									
d 0	11.6	11.6	0.17	0.565	11.6	11.6	11.6	0.17	0.837
d 10	13.4 <sup>x</sup>	13.6 <sup>y</sup>	0.17	0.063	13.5	13.4	13.6	0.18	0.379
d 24	24.2	24.6	0.30	0.179	24.0 <sup>x</sup>	24.4 <sup>xy</sup>	24.9 <sup>y</sup>	0.33	0.074
d 52	58.1	59.0	0.58	0.173	57.8	58.9	58.9	0.65	0.240
Economics, \$									
Feed cost/pig	10.73 <sup>a</sup>	11.45 <sup>b</sup>	0.075	0.024	10.83 <sup>x</sup>	11.02 <sup>x</sup>	11.28 <sup>y</sup>	0.091	0.078
Feed cost/lb gain <sup>3</sup>	0.233	0.242	0.001	0.445	0.237	0.234	0.241	0.002	0.183
Total revenue/pig <sup>4</sup>	24.91	25.35	0.244	0.194	24.70	25.40	25.29	0.292	0.188
IOFC <sup>5</sup>	14.18	14.00	0.181	0.820	13.87	14.38	14.00	0.217	0.216

<sup>1</sup> A total of 1,134 pigs (PIC 337 × 1050, initially 11.6 lb BW) were used in a 52-d growth trial with 27 pigs per pen and 7 pens per treatment.

<sup>2</sup> Corn was valued at \$3.60/bu (\$129/ton), DDGS at \$180/ton, soybean meal at \$354/ton, and AminoGut at \$2.34/lb.

<sup>3</sup> Feed cost/lb gain = total feed cost divided by total gain per pig. Cost per ton used not considering processing costs.

<sup>4</sup> One lb of live gain was considered to be worth \$0.68. Total revenue/pig = total gain/pig × \$0.54.

<sup>5</sup> Income over feed cost = total revenue/pig – feed cost/pig.

<sup>a,b</sup> Within rows and within each factor (protein source or AminoGut duration), means with different superscript differ (*P* < 0.05).

<sup>x,y,z</sup> Within rows and within each factor (protein source or AminoGut duration), means with different superscript differ (*P* < 0.10).

## Effect of Enzymatically Fermented Soybean Meal and *Lactobacillus Plantarum* on Nursery Pig Performance<sup>1,2</sup>

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### Summary

A total 360 pigs (PIC C-29 × 359, initially 12.2 lb) were used in a 45-d trial to determine the effects of enzymatically fermented soybean meal (EFS) and *Lactobacillus plantarum* (LP1) on nursery pig performance. Pigs were allotted by BW and sex, and randomly assigned to 1 of 4 dietary treatments, with 9 replications per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of added EFS (0 vs. 8% replacing soybean meal) and LP1 (0 vs. 0.1%). Experimental diets were fed in two phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 24) with a common diet fed to all pigs from d 24 to 45 post-weaning. From d 0 to 14, pigs fed diets containing EFS had decreased ( $P < 0.05$ ) ADG, ADFI, and d 14 BW compared with pigs fed diets without EFS. However, there were no differences in growth performance observed for LP1. From d 14 to 24, pigs fed diets containing EFS had improved ( $P = 0.035$ ) F/G; however, there were no differences in ADG or ADFI among treatments. Furthermore, no differences in growth performance were observed for LP1. From d 0 to 24, pigs fed the diet containing EFS had a tendency for decreased ( $P = 0.09$ ) ADFI compared to pigs fed diets without EFS; however, no differences were observed for ADG and F/G. In addition, pigs fed diets containing LP1 had a tendency for improved ( $P = 0.06$ ) F/G compared to pigs fed diets without LP1, but no differences were observed for ADG or ADFI. During the common period (d 24 to 45), there was a tendency for increased ( $P = 0.08$ ) ADFI for pigs previously fed diets containing LP1 compared to pigs previously fed diets without LP1; however, there were no differences detected for ADG or F/G. Overall (d 0 to 45), a LP1 × EFS interaction was detected for F/G ( $P < 0.01$ ) where LP1 and EFS individually each improved ( $P < 0.05$ ) F/G, but when combined, F/G was similar to the control diet. No differences were observed for the main effects of EFS or LP1. In conclusion, pigs fed EFS had decreased ADFI which led to lower growth rates immediately post-weaning. Interestingly, the addition of LP1 and EFS in nursery

<sup>1</sup> Appreciation is expressed to Dr. Jason Sewell and Terry Waugh, Nutraferma Inc., Sioux City, IA, and Brent Ratliff, Kindstrom-Schmoll Inc., Eden Prairie, MN, for their technical support and to Nutraferma Inc., Sioux City, IA, for their partial financial support.

<sup>2</sup> Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, for their technical support and expertise in conducting the experiment.

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diets improved F/G when fed independently from one another, but when combined, no growth benefit was reported.

Key words: enzymatically fermented soybean meal, *Lactobacillus plantarum*, nursery pig

## Introduction

Voluntary feed intake is often low and variable directly after weaning. As a result, research has focused on how nutritional stressors can be overcome to stimulate feed intake and subsequently increase performance (Pluske et al., 1997<sup>4</sup>). Thus, highly palatable and nutrient dense protein sources are commonly added to nursery diets to encourage feed intake. Traditionally, this has been accomplished with the addition of milk and animal-based by-products. However, concern of cost, availability, and bio-security concerns has led many producers to seek alternatives.

One product that has gained significant interest over the years is the use of enzymatically fermented soybean meal (EFS). Enzymatically fermented soybean meal is a product obtained from the fermentation of conventional soybean meal using a mixed culture of bacteria and fungus (Wang et al., 2014<sup>5</sup>). This process can effectively reduce the number of anti-nutritional factors associated with allergenic responses in weaned pigs as well as modify the amino acid profile via microbial synthesis (Hong et al., 2004<sup>6</sup>). Likewise, the use of probiotics has been a major focus within the swine industry in recent years. Probiotics can be defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001<sup>7</sup>). Therefore, it's been proposed that probiotics have the potential ability to influence the microbiota balance and the integrity of the intestinal epithelia (Metzler et al., 2005<sup>8</sup>). Thus, the objective of this study was to evaluate the growth performance of nursery pigs fed EFS and a commercially produced probiotic (*Lactobacillus plantarum*: LP1) independently and together in a commercial research facility.

## Procedures

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. Pens were 5 × 6 ft to allow 3 ft<sup>2</sup> per pig. Nursery rooms were not power washed or disinfected after the previous group of pigs.

<sup>4</sup> Pluske, J.R., D.J. Hampson, and I.H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51:215-236.

<sup>5</sup> Wang, Y., X.T. Liu, H.L. Wang, D.F. Li, X.S. Piao, and W.Q. Lu. 2014. Optimization of processing conditions for solid-state fermented soybean meal and its effects on growth performance and nutrient digestibility of weanling pigs. *Livest. Sci.* 170:91-99.

<sup>6</sup> Hong, K. J., C. H. Lee, and S. W. Kim. 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food* 7:430-434.

<sup>7</sup> FAO/WHO (Food and Agriculture Organization/World Health Organization) 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. <ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf>.

<sup>8</sup> Metzler, B., E. Bauer, R. Mosenthin. 2005. Microflora management in the gastrointestinal tract of piglets. *Asian-Aust. J. Anim. Sci.* 18:1353-1362.

A total of 360 pigs (PIC C-29 × 359, initially 12.2 lb) with 10 pigs per pen and 9 replications per treatment were used in a 45-d growth performance trial evaluating the effects of enzymatically fermented soybean meal and *Lactobacillus plantarum* supplementation on the growth performance of nursery pigs. Pigs were weaned at approximately 18 to 20 d and allotted to pens based on initial weight in a completely randomized design to 1 of 4 dietary treatments (Tables 1 and 2). Dietary treatments were arranged in a 2 × 2 factorial with main effects of added EFS (0 vs. 8% replacing soybean meal) and LP1 (0 vs. 0.1%). Pigs and feeders were weighed on d 0, 7, 14, 24, 35, and 45 of the trial to determine ADG, ADFI, and F/G.

Experimental diets were fed in two phases, with the first phase being provided at 5 lb per pig from d 0 to 14. The second phase was fed until pigs reached approximately 25 lb BW (d 24 post-weaning). A common nursery Phase 3 diet was then fed to all pigs for three weeks following the experimental diets (d 24 to 45 post-weaning). All diets were fed in pellet form during the trial.

Samples of treatment protein sources were collected at the feed mill during diet manufacturing. Complete diet samples were obtained from each dietary treatment each wk during the study and composited. Samples were then stored at -4°F until analysis. Composite samples of protein sources and diets were analyzed for DM, CP, ADF, NDF, crude fiber, Ca, P, Cl, Na, ether extract, and starch (Ward Laboratory, Kearney, NE).

Data were analyzed using the PROC GLIMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Dietary treatments were the fixed effect in the analysis. The main effects of LP1 and EFS, as well as their interactions, were tested. Differences between treatments were determined by using least square means, with results considered significant at a  $P$ -value  $\leq 0.05$  and considered a trend  $0.05 < P \leq 0.10$ .

## Results and Discussion

Chemical analysis of experimental diets and the EFS fed during this trial were reasonably consistent with formulated values.

There were no EFS × LP1 interactions observed for the entire study with the exception of overall (d 0 to 45) F/G. From d 0 to 14, pigs fed diets containing EFS had decreased ( $P < 0.05$ ) ADG, ADFI, and d 14 BW compared to pigs fed diets without EFS. Added LP1 had no effect on d 0 to 14 performance. From d 14 to 24, pigs fed diets containing EFS had improved ( $P = 0.035$ ) F/G; however, there were no differences in ADG or ADFI among treatments. Furthermore, no differences in growth performance were observed for LP1.

From d 0 to 24, pigs fed the diet containing EFS had a tendency for decreased ( $P = 0.09$ ) ADFI compared to pigs fed diets without EFS; however, no differences were observed for ADG and F/G. In addition, pigs fed diets containing LP1 had a tendency for improved ( $P = 0.06$ ) F/G compared to pigs fed diets without LP1, but no differences were observed for ADG and ADFI.

During the common period (d 24 to 45), there was a tendency for increased ( $P = 0.08$ ) ADFI for pigs previously fed diets containing LP1 compared to the negative control and negative control with EFS; however, there were no differences detected for ADG or F/G.

Overall (d 0 to 45), an LP1  $\times$  EFS interaction was detected for F/G ( $P < 0.01$ ) where LP1 and EFS each improved ( $P < 0.05$ ) F/G, but when combined, F/G was similar to the control diet. No differences were observed for the main effects of LP1 or EFS.

In conclusion, pigs fed EFS had poorer ADFI which led to poorer growth rates immediately post-weaning. Interestingly, the addition of LP1 and EFS in nursery diets improved F/G when fed independently from one another, but when combined, no growth benefit was reported. A possible explanation for the lack of response could be attributed to the fact that the EFS contained fewer anti-nutritional factors, thus potentially reducing gut inflammation and the opportunity for bacterial overgrowth that LP1 has been recognized to act upon. Nevertheless, the post-weaning period remains a challenge for newly weaned pigs that will continue to warrant research to evaluate specialty ingredients that can maximize feed intake while improving feed efficiency.

**Table 1. Chemical analysis of Phase 1 and 2 diets<sup>1,2</sup>**

Item, %	Control	Control +		
		LP1	EFS	LP1 + EFS
Phase 1 diets				
DM	89.87	90.34	91.86	91.52
CP	24.20	24.60	23.20	24.40
ADF	4.80	4.70	4.70	5.00
NDF	8.00	8.70	8.10	7.90
Crude fiber	2.80	3.40	2.60	2.70
Ca	0.93	0.93	1.02	0.88
P	0.72	0.75	0.65	0.65
Cl	0.58	0.66	0.66	0.64
Na	0.23	0.25	0.23	0.24
Ether extract	4.20	4.00	4.10	3.90
Ash	6.44	6.76	7.09	6.95
Phase 2 diets				
DM	90.07	90.39	89.70	90.73
CP	23.40	24.20	24.80	24.50
ADF	4.00	5.20	5.00	4.70
NDF	7.40	7.10	12.50	7.00
Crude fiber	2.80	2.80	3.90	2.80
Ca	0.87	0.86	0.81	0.83
P	0.57	0.66	0.67	0.58
Cl	0.62	0.64	0.61	0.51
Na	0.23	0.24	0.21	0.23
Ether extract	4.50	4.70	4.60	4.60
Ash	6.34	6.25	5.75	6.21

<sup>1</sup> Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of the diets were then submitted to Ward Laboratories, Inc. (Kearny, NE) for analysis.

<sup>2</sup> *Lactobacillus plantarum* and enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).

**Table 2. Chemical analysis of the Phase 3 diet<sup>1</sup>**

Item, %	Common diet
DM	88.40
CP	22.90
ADF	6.50
NDF	13.40
Crude fiber	3.40
Ca	0.92
P	0.70
Cl	0.26
Na	0.19
Ether extract	4.90
Ash	5.24

<sup>1</sup>A composite sample was submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis.

**Table 3. Laboratory analysis of enzymatically fermented soybean meal<sup>1</sup>**

Item, %	EFS <sup>2</sup>
DM	94.56
CP	51.00
ADF	10.10
NDF	12.70
Crude fiber	3.80
Ca	0.60
P	0.77
Cl	0.63
Na	0.03
Ether extract	1.50
Ash	6.94

<sup>1</sup>Proximate analysis for enzymatically fermented soybean meal was analyzed by Ward Labs, Kearney, NE.

<sup>2</sup>Enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).

**Table 4. Ingredient composition of experimental diets<sup>1</sup>**

Ingredient, %	Phase 1		Phase 2		Phase 3
	Control	EFS	Control	EFS	Common
Corn <sup>2</sup>	28.00	28.59	38.09	38.61	52.02
Soybean meal, 46.5% CP	35.03	26.50	36.00	27.50	32.50
Corn DDGS <sup>3</sup>	10.00	10.00	10.00	10.00	10.00
Spray dried whey	21.75	21.75	10.85	10.85	---
EFS <sup>4</sup>	---	8.00	---	8.00	---
Tallow	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.05	1.10	1.15
Monocalcium P, 21% P	0.85	0.75	0.75	0.65	1.10
Salt	0.25	0.25	0.30	0.30	0.40
L-Lys HCL	0.24	0.28	0.23	0.27	0.37
DL-Met	0.15	0.15	0.12	0.12	0.14
L-Thr	0.09	0.09	0.09	0.09	0.15
L-Trp	---	---	---	---	0.01
Phytase <sup>5</sup>	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.40	0.40	0.26	0.26	---
Choline chloride, 70% liq.	0.04	0.04	0.04	0.04	---
Selenium, 0.6%	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09	0.09	0.09
Vitamin premix	0.10	0.10	0.10	0.10	0.05
TOTAL	100	100	100	100	100

*continued*

**Table 4. Ingredient composition of experimental diets<sup>1</sup>**

	Phase 1		Phase 2		Phase 3
	Control	EFS	Control	EFS	Common
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.40	1.40	1.35	1.35	1.30
Met:Lys	34	35	33	33	35
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	65	65	65	65	65
Trp:Lys	20	20	20	20	18
Val:Lys	71	71	73	73	69
ME, kcal/lb	1,520	1,524	1,519	1,519	1,515
CP, %	24.55	24.80	24.57	24.83	22.91
Ca, %	0.96	0.96	0.90	0.90	0.92
P, %	0.86	0.84	0.80	0.80	0.81
Available P, %	0.59	0.59	0.50	0.50	0.50

<sup>1</sup>Phase 1 diets were fed for 14 d or to approximately 15 lb BW (5 lb/pig). Phase 2 diets were fed from approximately 15 lb to approximately 25 lb BW. Phase 3 diets were fed d 24 to 45 post-weaning.

<sup>2</sup>*Lactobacillus plantarum* (Nutraferma, Sioux City, IA) was included in the diet at 0.10% at the expense of corn.

<sup>3</sup>Dried distillers grains with solubles.

<sup>4</sup>Enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).

<sup>5</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of diet, with a release of 0.13% available P.

**Table 5. Effect of enzymatically fermented soybean meal and *Lactobacillus plantarum* on nursery pig performance**

	Control <sup>2</sup>	Negative Control +			SEM	Probability, <i>P</i> <
		LP1 <sup>3</sup>	EFS <sup>3</sup>	LP1 + EFS		LP1 × EFS
BW, lb						
d 0	12.2	12.2	12.2	12.2	0.03	0.262
d 14	16.8	16.9	16.5	16.5	0.17	0.814
d 24	24.0	24.3	23.9	24.2	0.30	0.892
d 45	50.2	51.6	50.9	51.3	0.61	0.460
d 0 to 14						
ADG, lb	0.33	0.34	0.31	0.30	0.012	0.617
ADFI, lb	0.37	0.35	0.34	0.34	0.009	0.269
F/G	1.14	1.04	1.11	1.12	0.032	0.124
d 14 to 24						
ADG, lb	0.72	0.75	0.74	0.77	0.023	0.943
ADFI, lb	1.08	1.08	1.05	1.08	0.022	0.595
F/G	1.52	1.46	1.43	1.41	0.032	0.534
d 0 to 24						
ADG, lb	0.49	0.51	0.49	0.50	0.013	0.696
ADFI, lb	0.67	0.66	0.64	0.64	0.012	0.588
F/G	1.36	1.29	1.31	1.30	0.021	0.174
d 24 to 45						
ADG, lb	1.25	1.30	1.28	1.29	0.020	0.375
ADFI, lb	1.79	1.84	1.79	1.85	0.030	0.928
F/G	1.43	1.42	1.40	1.43	0.014	0.113
d 0 to 45						
ADG, lb	0.85	0.88	0.86	0.87	0.014	0.366
ADFI, lb	1.19	1.21	1.18	1.21	0.019	0.837
F/G	1.41 <sup>a</sup>	1.38 <sup>b</sup>	1.37 <sup>b</sup>	1.39 <sup>ab</sup>	0.010	0.007

<sup>ab</sup> Means within the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup> A total of 360 pigs (PIC C-29 × 359) were used with 10 pigs/pen and 9 replications/trt.

<sup>2</sup> Negative control – high soybean meal; first 24 d; Phases 1 and 2.

<sup>3</sup> *Lactobacillus plantarum* (fed from d 0 to 24) and EFS (fed from d 0 to 24) (Nutraferma, Sioux City, IA).

**Table 6. Main Effects of enzymatically fermented soybean meal and *Lactobacillus plantarum* on nursery pig performance<sup>1</sup>**

	LP1 <sup>2</sup>		EFS <sup>2</sup>		SEM	Probability, <i>P</i> <	
	-	+	-	+		LP1	EFS
BW, lb							
d 0	12.2	12.2	12.2	12.2	0.02	0.918	0.424
d 14	16.6	16.7	16.8	16.5	0.12	0.728	0.046
d 24	24.0	24.3	24.1	24.1	0.21	0.246	0.757
d 45	50.5	51.5	50.9	51.1	0.43	0.131	0.785
d 0 to 14							
ADG, lb	0.32	0.32	0.34	0.31	0.009	0.891	0.026
ADFI, lb	0.36	0.35	0.36	0.34	0.006	0.179	0.013
F/G	1.13	1.08	1.08	1.12	0.023	0.159	0.420
d 14 to 24							
ADG, lb	0.73	0.76	0.73	0.76	0.016	0.197	0.269
ADFI, lb	1.07	1.08	1.08	1.07	0.015	0.595	0.434
F/G	1.48	1.43	1.49	1.42	0.023	0.172	0.035
d 0 to 24							
ADG, lb	0.49	0.50	0.50	0.49	0.009	0.365	0.634
ADFI, lb	0.65	0.65	0.66	0.64	0.009	0.786	0.092
F/G	1.33	1.30	1.33	1.30	0.015	0.058	0.246
d 24 to 45							
ADG, lb	1.27	1.30	1.28	1.29	0.014	0.174	0.608
ADFI, lb	1.79	1.85	1.82	1.82	0.022	0.075	0.871
F/G	1.41	1.43	1.42	1.41	0.010	0.291	0.504
d 0 to 45							
ADG, lb	0.85	0.87	0.86	0.86	0.010	0.150	0.969
ADFI, lb	1.18	1.21	1.20	1.19	0.013	0.174	0.703
F/G	1.39	1.39	1.39	1.38	0.007	0.820	0.147

<sup>1</sup> A total of 360 pigs (PIC C-29 × 359) were used for the study.

<sup>2</sup> Negative control – high soybean meal; first 24 d; Phases 1 and 2.

<sup>3</sup> *Lactobacillus plantarum* (fed from d 0 to 24) and enzymatically fermented soybean meal (fed from d 0 to 24) (Nutraferma, Sioux City, IA).

## Effect of Feeding Varying Levels of *Lactobacillus Plantarum* on Nursery Pig Performance<sup>1,2</sup>

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### Summary

A total of 360 pigs (PIC C-29 × 359, initially 13.1 lb BW) were used in a 42-d growth performance trial evaluating the effects of feeding varying levels of *Lactobacillus plantarum* on nursery pig performance. Pigs were allotted by BW and sex, and randomly assigned to 1 of 4 dietary treatments in a completely randomized design. Experimental diets were fed in three phases (Phase 1, d 0 to 7; Phase 2, d 7 to 21, and Phase 3, d 21 to 42). Treatment diets were formulated to include 0, 0.05, 0.10, or 0.20% *Lactobacillus plantarum* product (LP1; Nutraferma Inc., Sioux City, IA). *Lactobacillus plantarum* is a facultative heterofermentative plant-associated lactic acid bacterium that is tolerant against bile salts and low pH, improving survivability in the GIT (de Vries et al., 2006; da Silva Sabo et al., 2014).<sup>4,5</sup> All experimental diets were pelleted. During Phase 1 and 2, there were no differences in growth performance among dietary treatment. During Phase 3, ADG and ADFI were not influenced by treatment; however, increasing LP1 tended to improve F/G (quadratic,  $P = 0.085$ ) up to the 0.10% level. Overall (d 0 to 42), no differences in growth performance were observed among dietary treatments. In conclusion, increasing dietary levels of LP1 did not impact nursery pig performance.

Key words: *Lactobacillus plantarum*, growth performance, nursery pig, probiotic

### Introduction

Growth promotional feed additives continue to be an area of emphasis for evaluation, especially in nursery pig diets. One of the classes of feed additives that has gained sig-

<sup>1</sup> Appreciation is expressed to Dr. Jason Sewell and Terry Waugh, Nutraferma Inc., Sioux City, IA and Brent Ratliff, Kindstrom-Schmoll Inc., Eden Prairie, MN for their technical support and to Nutraferma Inc., Sioux City, IA for their financial support.

<sup>2</sup> Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, for their technical support and expertise in conducting the experiment.

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<sup>4</sup> de Vries, M. C., E. E. Vaughan, M. Kleerebezem, W. M. de Vos. 2006. *Lactobacillus plantarum* – survival, functional and potential probiotic properties in the human intestinal tract.

<sup>5</sup> da Silva Sabo, S., M. Vitolo, J. M. Domínguez González, and R. P. d. S. Oliveira. 2014. Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. Food Res. Int. 64: 527-536.

nificant interest is probiotics. Probiotics can be defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).<sup>6</sup> Mechanistically, the modes of action of probiotics are likely to include competitive exclusion of pathogenic bacteria, regulation of local cell-mediated immune responses, increasing antibody production, promotion of epithelial barrier integrity, and the reduction of epithelial cell apoptosis (Ng et al., 2009).<sup>7</sup> However, debate still remains within the scientific community on these modes of actions.

Among the diverse bacterial species used for probiotics, the nonpathogenic class of *Bacillus* species are some of the most extensively studied. Of the species, *L. plantarum* has shown some of the more promising beneficial results on the overall gastrointestinal microbiota of nursery pigs (Pieper et al., 2009; Guerra-Ordaz et al., 2013).<sup>8,9</sup> *Lactobacillus plantarum* is a facultative heterofermentative plant-associated lactic acid bacterium that is tolerant against bile salts and low pH, improving survivability in the GIT (de Vries et al., 2006; da Silva Sabo et al., 2014).<sup>10,11</sup> These findings certainly make it a promising bacterial source that could potentially be used in swine diets. However, in the context of these studies, all research has been carried out under highly controlled environments. Research examining its impact in commercial settings is scarce. Thus, the objective of this study was to evaluate the efficacy of *Lactobacillus plantarum* in nursery pigs in a commercial research facility.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. Pens were 5 × 6 ft to allow 3 ft<sup>2</sup> per pig. Nursery rooms were not power washed or disinfected after the previous 6 groups of pigs.

A total of 360 pigs (PIC C-29 × 359, initially 13.1 lb BW) with 10 pigs per pen and 9 replications per treatment were used in a 42-d trial. Pigs were weaned at approximately 16 to 20 d of age and allotted to pens based on initial BW and gender to 1 of 4 dietary

<sup>6</sup> FAO/WHO (Food and Agriculture Organization/World Health Organization) 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. <ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf>.

<sup>7</sup> Ng, S. C., A. L. Hart, M. A. Kamm, A. J. Stagg, S. C. Knight. 2009. Mechanisms of action of probiotics: Recent advances. *Inflamm. Bowel Dis.* 15: 300-310.

<sup>8</sup> Pieper, R., P. Janczyk, V. Urubshurov, U. Korn, B. Pieper, and W. B. Souffrant. 2009. Effect of a single administration of *Lactobacillus plantarum* DSMZ 8862/8866 before and at the time point of weaning on intestinal microbial communities in piglets. *Int. J. Microbiol.* 130: 215-236.

<sup>9</sup> Guerra-Ordaz, A. A., G. Gonzalez-Ortiz, R. M. La Ragione, M. J. Woodward, J. W. Collins, J. F. Perez, S. M. Martin-Orue. 2013. Effect of inclusion of lactulose and *Lactobacillus plantarum* on the intestinal environment and performance of piglets at weaning. *Anim. Feed Sci. Tech.* 185: 160-168.

<sup>10</sup> de Vries, M. C., E. E. Vaughan, M. Kleerebezem, W. M. de Vos. 2006. *Lactobacillus plantarum* – survival, functional and potential probiotic properties in the human intestinal tract.

<sup>11</sup> da Silva Sabo, S., M. Vitolo, J. M. Domínguez González, and R. P. d. S. Oliveira. 2014. Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. *Food Res. Int.* 64: 527-536.

treatments in a completely randomized design. Pigs and feeders were weighed every 7 d of the trial to determine ADG, ADFI, and F/G.

Experimental diets were fed in three phases (Table 1). Phase 1 was fed from d 0 to 7 d. The second phase was fed from d 7 to 21 (~ 25 lb BW), while phase 3 was fed from d 21 to 42 post-weaning. Treatment diets were formulated to include 0, 0.05, 0.10, or 0.20% of *Lactobacillus plantarum* product (LP1; Nutraferma Inc., Sioux City, IA). All treatment diets within phase were formulated to similar nutrient levels. All experimental diets were fed in pellet form.

Complete diet samples were collected and analyzed for DM, CP, crude fat, Ca, and P (Table 2).

Data were analyzed using the PROC GLIMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Dietary treatment served as the fixed effect in the model. Linear and quadratic responses were determined for increasing LP1. A  $P$  value  $\leq 0.05$  was considered significant and  $0.05 < P \leq 0.10$  was considered a tendency.

## Results and Discussion

Chemical analysis of complete diets revealed that analyzed values were similar to calculated values (Table 2).

From d 0 to 7 (Phase 1) and d 7 to 21 (Phase 2), there were no differences in growth performance observed among dietary treatments (Table 3). From d 21 to 42 (Phase 3), ADG and ADFI were not influenced by treatment; however, F/G tended to improve (quadratic,  $P = 0.085$ ) with increasing LP1 up to 0.10%. Overall (d 0 to 42), there were no differences in growth performance detected between dietary treatments.

The addition of up to 0.20% LP1 used in this study, resulted in no improvements in overall ADG, ADFI, or F/G. Additional research should be conducted to determine if LP1 elicits a response in other diet formulation or health statuses. One possible explanation could be attributed to the fact that the overall health status of the pigs throughout this experiment was very good. This occurred although the room that they were placed into had not been power washed or disinfected after the previous 6 groups of pigs.

**Table 1. Phase 1, 2, 3 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	35.67	41.45	52.01
Soybean meal, 46.5% CP	30.00	30.00	32.54
Corn DDGS <sup>2</sup>	5.00	10.00	10.00
Spray dried whey	21.74	10.87	---
Fish meal	2.50	3.00	---
Tallow	2.00	2.00	2.00
Limestone	1.06	0.93	1.13
Monocalcium P, 21% P	0.80	0.40	1.09
Sodium chloride	0.25	0.30	0.40
L-lysine HCl	0.22	0.28	0.37
DL-methionine	0.15	0.12	0.14
L-threonine	0.09	0.11	0.15
L-tryptophan	0.01	0.02	0.01
Quantum Blue <sup>3</sup>	0.01	0.01	0.01
Zinc oxide	0.26	0.26	---
Choline chloride, 70% liq.	0.04	0.04	---
Selenium, 0.06%	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09
Vitamin premix	0.10	0.10	0.05
LP1 <sup>4</sup>	---	---	---
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.40	1.35	1.30
Met:Lys	33	35	35
Met and Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	20	20	18
Val:Lys	70	71	69
ME, kcal/lb	1,530	1,532	1,515
CP, %	23.36	23.92	22.92
Ca, %	0.96	0.91	0.91
P, %	0.85	0.78	0.81
Available P, %	0.59	0.50	0.50

<sup>1</sup>Phase 1 diets were fed from d 0 to 7 (~13.1 to 14 lb BW), Phase 2 diets from d 7 to 21 (~14 to 24 lb BW) and Phase 3 diets from d 21 to 42 (~24 to 51 lb BW).

<sup>2</sup>Dried distillers grains with solubles.

<sup>3</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of diet, with a release of 0.13% available P.

<sup>4</sup>*Lactobacillus plantarum* (LP1; Nutraferma Inc., Sioux City, IA) was substituted at 0.05, 0.10, and 0.20% of the diet at the expense of corn.

**Table 2. Laboratory analysis of Phases 1, 2, and 3 experimental diets<sup>1</sup>**

Item, %	Control	0.05%	LPI <sup>2</sup>	
			0.10%	0.20%
Phase 1 diets				
DM	89.68	90.20	90.40	90.66
CP	22.40	22.10	23.20	21.20
Crude fat	4.90	4.70	4.80	4.70
Ca	0.80	0.84	0.75	0.77
P	0.71	0.72	0.70	0.66
Phase 2 diets				
DM	89.87	89.09	88.95	89.53
CP	22.40	21.30	22.40	23.10
Crude fat	5.60	5.30	5.70	5.70
Ca	0.75	0.77	0.78	0.75
P	0.66	0.66	0.64	0.63
Phase 3 diets				
DM	88.35	88.27	88.75	89.16
CP	20.40	22.10	22.80	22.50
Crude fat	5.30	5.20	5.40	5.20
Ca	0.73	0.74	0.72	0.71
P	0.67	0.67	0.68	0.70

<sup>1</sup>Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of diets were then submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, crude fat, Ca, and P.

<sup>2</sup>*Lactobacillus plantarum* (Nutraferma, Sioux City, IA).

**Table 3. Effect of feeding varying levels of *Lactobacillus plantarum* on nursery pig performance<sup>1</sup>**

Diets	Control	LP1 <sup>2</sup>			SEM	Probability, <i>P</i> <	
		0.05%	0.10%	0.20%		Linear	Quadratic
BW, lb							
d 0	13.1	13.1	13.1	13.1	0.02	0.616	0.455
d 7	14.1	14.0	14.0	14.1	0.10	0.962	0.394
d 21	24.6	24.1	24.1	24.3	0.31	0.601	0.178
d 42	51.9	51.5	51.1	51.1	0.66	0.402	0.612
d 0 to 7							
ADG, lb	0.15	0.13	0.14	0.14	0.014	0.894	0.456
ADFI, lb	0.23	0.22	0.22	0.23	0.010	0.955	0.321
F/G	1.58	1.98	1.78	1.68	0.194	0.986	0.268
d 7 to 21							
ADG, lb	0.75	0.72	0.71	0.73	0.019	0.606	0.209
ADFI, lb	0.90	0.88	0.85	0.87	0.020	0.276	0.260
F/G	1.20	1.23	1.19	1.19	0.017	0.326	0.611
d 21 to 42							
ADG, lb	1.30	1.30	1.29	1.28	0.021	0.405	0.919
ADFI, lb	1.81	1.79	1.77	1.78	0.029	0.451	0.442
F/G	1.40	1.38	1.37	1.39	0.011	0.898	0.085
d 0 to 42							
ADG, lb	0.92	0.91	0.90	0.90	0.015	0.508	0.567
ADFI, lb	1.24	1.22	1.20	1.22	0.020	0.448	0.316
F/G	1.35	1.34	1.33	1.34	0.010	0.783	0.314

<sup>1</sup>A total of 360 pigs (PIC C-29 × 359) were used in a 3-phase nursery trial with 10 pigs per pen and 9 replications per treatment. All experimental diets were fed in three phases (d 0 to 7, d 7 to 21, and d 21 to 42).

<sup>2</sup>*Lactobacillus plantarum* (fed from d 0 to 42) (Nutraferma, Sioux, City, IA).

## Effect of Diet Complexity and Specialty Protein Source on Nursery Pig Performance<sup>1,2</sup>

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### Summary

A total of 720 nursery pigs (PIC C-29 × 359, initially 12.5 lb BW) were used in a 42-d growth trial to determine the effects of diet complexity and specialty soy protein source on nursery pig performance. Pigs were allotted by BW and sex, and randomly assigned to 1 of 6 dietary treatments in a 2 × 3 factorial arrangement with main effects of diet complexity (complex vs. simple) and specialty protein source (fish meal, HP 300, or HP 800). The HP 300 and HP 800 are two different enzymatically treated soy products manufactured and sold by Hamlet Protein (Findlay, OH). Experimental diets were fed in two phases (Phase 1 was budgeted at 5 lb per pig and Phase 2 was fed thereafter until d 21) with a common diet fed for 3 wk following the experimental diets. No interactions were observed between diet complexity and protein source for growth performance for any phase or overall. From d 0 to 7, pigs fed the complex diet had a tendency for improved ADG ( $P = 0.078$ ) and d 7 BW ( $P = 0.053$ ) compared to pigs fed the simple diet. There was no difference in performance observed from d 7 to 21; however, for the overall treatment feeding period (d 0-21), pigs fed the complex diets had improved F/G ( $P = 0.037$ ) compared to pigs fed the simple diets. During the Phase 3 common diet feeding period (d 21 to 42), no differences were observed between pigs previously fed different diet complexity or protein sources. Overall (d 0 to 42), no differences in growth performance were found between treatments. For economics, pigs fed a simple diet tended to have greater IOFC ( $P = 0.055$ ). Feed cost per pound of gain was lower ( $P = 0.002$ ) for pigs fed diets with HP 300 and HP 800 compared to those fed diets with fish meal. In summary, this study suggests that the differences in diet complexity used in this study had minor impacts on growth performance during the phases in which they were fed but not overall. Furthermore, the three specialty protein sources used in this study resulted in similar growth performance.

Key words: diet complexity, nursery pig, protein sources

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<sup>2</sup> Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, for their technical support and expertise in conducting the experiment.

<sup>3</sup> Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.

## Introduction

Soybean meal is one of the most readily available and economical protein sources commonly fed to pigs. Due to a number of anti-nutritional factors, its inclusion in newly weaned pig diets has been limited. Thus, specialty animal proteins, such as animal plasma, blood cells, or fish meal have been commonly added as highly digestible amino acid sources in starter diets. In recent years, the cost and variability of specialty animal proteins has increased while availability of some sources has decreased. Furthermore, biosecurity concerns have led some nutritionists to remove any porcine-derived products from swine diets. As a result, producers have sought more economical and readily available alternatives.

One category of alternative protein sources that has gained significant interest in recent years is further processed soybean meal products. Their benefits include lower levels of common anti-nutritional factors compared to conventional soybean meal (Cervantes-Pahm and Stein, 2010;<sup>4</sup> Goebel and Stein, 2011<sup>5</sup>) as well as a greater concentration of digestible AA (Cervantes-Pahm and Stein, 2010<sup>4</sup>). One such product is HP 300 (Hamlet Protein, Findlay, OH), which is a finely ground hydrolyzed soy protein produced from conventional soybean meal treated to remove anti-nutritional factors (Cervantes-Pahm and Stein, 2010;<sup>4</sup> Goebel and Stein, 2011<sup>5</sup>). Recently, the same supplier has also introduced another further-processed soybean meal product (HP 800), but minimal data are available to determine its effects on growth performance of weanling pigs. Thus, the objective of this study was to compare the performance of nursery pigs fed different protein sources in varying diet complexities in a commercial research setting.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. Pens were 5 × 6 ft to allow 3 ft<sup>2</sup> per pig. Nursery rooms were not power washed or disinfected after the previous group of pigs.

A total of 720 pigs (PIC C-29 × 359, initially 12.5 lb) with 10 pigs per pen and 12 replications per treatment were used in a 42-d growth performance trial evaluating the effects of diet complexity (complex vs. simple) and protein source (fish meal, HP 300, or HP 800) on the growth performance of nursery pigs. Pigs were weaned at approximately 18 to 20 d and allotted to pens based on initial BW and gender to 1 of 6 treatments in a completely randomized block design. Pigs and feeders were weighed every 7 d of the trial to determine ADG, ADFI, and F/G.

Experimental diets (Tables 1 and 2) were fed in two phases, with the first phase being provided at 5 lb per pig. The second phase was fed until pigs reached approximately 25 lb BW (d 21 post-weaning). The complex diet contained 20% and 10% lactose,

<sup>4</sup> Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented and enzyme-treated soybean meal and in soy protein. *J. Anim. Sci.* 88:2674-2683.

<sup>5</sup> Goebel, K. P., and H. H. Stein. 2011. Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs. *J. Anim. Sci.* 89:764-772.

while the simple diet contained 12% and 5% lactose in Phases 1 and 2, respectively. To maintain equal soybean meal and SID lysine levels across treatments within phase, fish meal, HP 300, and HP 800 were adjusted accordingly. In addition, the complex diet contained oat meal, Tak-Tik flavoring (Pancosma, Geneva, Switzerland), BioPlus2B (Chr. Hansen BioSystems, Hoersholm, Denmark), and KemGest (Kemin Industries, Des Moines, IA) in both Phases 1 and 2. A common diet was fed for 3 wk following the treatment diets (d 21 to 42; Table 3). The common diet formulated for this trial was a standard nursery diet fed in commercial production. All experimental diets were fed in pellet form.

Samples of the protein sources were collected at the feed mill during diet manufacture. Complete diet samples were obtained from each dietary treatment each wk during the study and composited. Composite samples of protein sources and diets were analyzed for DM, CP, ADF, NDF, crude fiber, Ca, P, Cl, salt, ether extract, and starch (Ward Laboratory, Kearney, NE).

An economic analysis performed at the conclusion of the trial determined the financial impact of diet type and protein sources. For all economic calculations, ingredient prices for June 2015 were used, with corn valued at \$3.58/bu (\$141/ton), soybean meal at \$397/ton, DDGS at \$158/ton, lactose at \$600/ton, fish meal at \$1,992/ton, HP 300 at \$930/ton and HP 800 at \$1,004/ton. The total feed cost per pig was calculated by multiplying the ADFI by the diet cost and the number of days it was fed for the respective period. Cost per pound of gain was calculated by dividing the total feed cost per pig by the overall pounds gained. Revenue per pig was calculated by multiplying ADG times the total days in the trial times an assumed live price of \$65.00 per cwt. To calculate IOFC, total feed cost was subtracted from revenue per pig.

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Dietary treatments were the fixed effect and block and room served as the random effect in the analysis. A  $P$ -value  $\leq 0.05$  was considered significant and  $0.05 < P \leq 0.10$  was considered a tendency.

## Results and Discussion

Complete diet and protein source analyses (Tables 4 and 5) were similar to formulated values.

No interactions were observed between diet complexity and protein source (Table 6) or differences between specialty protein source (Table 8) for growth performance for any phase or overall. From d 0 to 7, pigs fed the complex diet had a tendency for improved ADG ( $P = 0.078$ ) and d 7 BW ( $P = 0.053$ ) compared to pigs fed the simple diet (Table 7). During Phase 2, (d 7 to 21), there were no differences in growth performance found between treatment diets. From d 0 to 21, pigs fed the complex diets had improved F/G ( $P = 0.037$ ) compared to pigs fed the simple diets.

During Phase 3 when a common diet was fed (d 21 to 42), no differences were observed between pigs previously fed different diet complexity or protein sources. Overall (d 0 – 42), no differences in growth performance were observed between treatments.

For the economic analysis, feed cost per pig and cost per pound of gain increased ( $P < 0.01$ ) for pigs fed a complex diet; however, no differences were detected for revenue per pig. As a result, IOFC tended to be lower ( $P = 0.055$ ; \$0.48/pig) for pigs fed the complex diets. Feed cost per pound of gain was lower ( $P = 0.002$ ) for pigs fed diets with HP 300 and HP 800 compared to those fed diets with fish meal; however, no differences were observed between protein sources for revenue per pig or IOFC.

In conclusion, regardless of the specialty protein and diet complexity used in this study, overall, pigs performed similarly during the trial. While minor differences were detected in diet complexity when fed, a greater magnitude was expected due to changes in lactose level and other ingredients used in the complex diet. One possible explanation for the lack of response observed could be attributed to the low feed intake and growth across all treatments for the first 7 d. The low feed intake might be indicative of an unknown health challenge or an ingredient quality issue such as the DDGS used. Additional research is warranted to confirm the responses observed in this experiment.

**Table 1. Phase 1 diet composition (as fed basis)<sup>1</sup>**

Ingredient, %	Complex			Simple		
	Fish meal	HP 300	HP 800	Fish meal	HP 300	HP 800
Corn	23.80	20.00	19.85	42.00	38.25	38.10
Soybean meal, 46.5% CP	21.50	21.50	21.50	21.50	21.50	21.50
Lactose	20.00	20.00	20.00	12.00	12.00	12.00
Corn DDGS <sup>2</sup>	10.00	10.00	10.00	10.00	10.00	10.00
Oat meal	10.00	10.00	10.00	---	---	---
Spray dried plasma	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.76	1.22	1.21	0.75	1.22	1.20
Monocalcium P, 21% P	0.44	1.25	1.25	0.46	1.28	1.28
Sodium chloride	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.35	0.36	0.36	0.36	0.37	0.37
DL-Met	0.17	0.18	0.18	0.15	0.17	0.16
L-Thr	0.17	0.14	0.15	0.17	0.14	0.14
L-Trp	0.05	0.02	0.02	0.06	0.03	0.02
Phytase <sup>3</sup>	0.01	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40
Choline chloride, 70% liq.	0.04	0.04	0.04	0.04	0.04	0.04
Selenium, 0.6%	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix	0.10	0.10	0.10	0.10	0.10	0.10
Tak-Tik <sup>4</sup>	0.02	0.02	0.02	---	---	---
Bioplus 2B <sup>5</sup>	0.01	0.01	0.01	---	---	---
Kem-gest <sup>6</sup>	0.20	0.20	0.20	---	---	---
Fish meal	7.75	---	---	7.75	---	---
HP 300 <sup>7</sup>	---	10.25	---	---	10.25	---
HP 800 <sup>7</sup>	---	---	10.45	---	---	10.45
Total	100	100	100	100	100	100

*continued*

**Table 1. Phase 1 diet composition (as fed basis)<sup>1</sup>**

Ingredient, %	Complex			Simple		
	Fish meal	HP 300	HP 800	Fish meal	HP 300	HP 800
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	1.40	1.40	1.40	1.40	1.40	1.40
Met:Lys	37	35	34	37	34	34
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	65	65	65	65	65	65
Trp:Lys	20	20	20	20	20	20
Val:Lys	65	69	68	67	71	69
ME, kcal/lb	1,582	1,573	1,573	1,568	1,558	1,558
CP, %	23.10	23.70	23.70	23.30	23.80	23.80
Ca, %	0.96	0.96	0.96	0.96	0.96	0.96
P, %	0.82	0.84	0.84	0.83	0.85	0.85
Available P, %	0.59	0.59	0.59	0.59	0.59	0.59

<sup>1</sup>Phase 1 diets were fed from weaning to approximately 15 lb BW (5 lb/pig).

<sup>2</sup>Dried distillers grains with solubles.

<sup>3</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of diet, with a release of 0.13% available P.

<sup>4</sup>Pancosma, Geneva, Switzerland.

<sup>5</sup>Chr. Hansen BioSystems, Hoersholm, Denmark.

<sup>6</sup>Kemin Industries, Des Moines, IA.

<sup>7</sup>Hamlet Protein, Findlay, OH.

**Table 2. Phase 2 diet composition (as fed basis)<sup>1</sup>**

Ingredient, %	Complex			Simple		
	Fish meal	HP 300	HP 800	Fish meal	HP 300	HP 800
Corn	34.18	31.06	30.93	49.39	46.23	46.10
Soybean meal, 46.5% CP	25.00	25.00	25.00	25.00	25.00	25.00
Lactose	10.00	10.00	10.00	5.00	5.00	5.00
Corn DDGS <sup>2</sup>	10.00	10.00	10.00	10.00	10.00	10.00
Oat meal	10.00	10.00	10.00	---	---	---
Spray dried plasma	---	---	---	---	---	---
Tallow	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.83	1.19	1.18	0.83	1.19	1.18
Monocalcium P, 21% P	0.33	0.97	0.96	0.37	1.00	1.00
Sodium chloride	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys	0.38	0.38	0.38	0.39	0.39	0.39
DL-Met	0.15	0.17	0.15	0.15	0.16	0.15
L-Thr	0.18	0.15	0.16	0.18	0.15	---
L-Trp	0.02	---	---	0.03	---	---
Phytase <sup>3</sup>	0.01	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.27	0.27	0.27	0.27	0.27	0.27
Selenium, 0.6%	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix	0.13	0.13	0.13	0.13	0.13	0.13
Tak-Tik <sup>4</sup>	0.02	0.02	0.02	---	---	---
Bioplus 2B <sup>5</sup>	0.01	0.01	0.01	---	---	---
Kem-Gest <sup>6</sup>	0.20	0.20	0.20	---	---	---
Fish meal	6.00	---	---	6.00	---	---
HP 300 <sup>7</sup>	---	8.21	---	---	8.20	---
HP 800 <sup>7</sup>	---	---	8.35	---	---	8.37
Total	100	100	100	100	100	100

*continued*

**Table 2. Phase 2 diet composition (as fed basis)<sup>1</sup>**

Ingredient, %	Complex			Simple		
	Fish meal	HP 300	HP 800	Fish meal	HP 300	HP 800
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Met:Lys	38	36	36	38	36	35
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	65	65	65	65	65	65
Trp:Lys	18	18	18	18	18	18
Val:Lys	65	69	67	67	70	69
ME, kcal/lb	1,557	1,550	1,550	1,548	1,541	1,541
CP, %	23.1	23.6	23.6	22.9	23.5	23.5
Ca, %	0.91	0.91	0.91	0.91	0.91	0.91
P, %	0.82	0.84	0.84	0.83	0.85	0.85
Available P, %	0.59	0.59	0.59	0.59	0.59	0.59

<sup>1</sup>Phase 2 diets were fed from 15 lb to approximately 25 lb BW.

<sup>2</sup>Dried distillers grain with solubles.

<sup>3</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of feed, with a release of 0.13% available P.

<sup>4</sup>Pancosma, Geneva, Switzerland.

<sup>5</sup>Chr. Hansen BioSystems, Hoersholm, Denmark.

<sup>6</sup>Kemin Industries, Des Moines, IA.

<sup>7</sup>Hamlet Protein, Findlay, OH.

**Table 3. Phase 3 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Common diet
Corn	61.15
Soybean meal, 46.5% CP	32.65
Tallow	2.50
Limestone	1.05
Monocalcium P, 21% P	0.82
Sodium chloride	0.50
L-lysine HCl	0.35
DL-methionine	0.16
L-threonine	0.14
Selenium, 0.6%	0.02
Trace mineral premix	0.05
Vitamin premix	0.09
Ameribond (2X) <sup>2</sup>	0.40
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lys	1.25
Met:lys	35
Met and cys:lys	58
Thr:lys	63
Trp:lys	18
Val:lys	66
ME, kcal/lb	1,523
CP, %	21.0
Ca, %	0.69
P, %	0.72
Available P, %	0.40

<sup>1</sup>Phase 3 diet was fed from 25 lb to approximately 50 lb BW.

<sup>2</sup>Borregaard LignoTech, Sarpsborg, Norway.

**Table 4. Laboratory analysis of Phases 1 and 2 experimental diets<sup>1,2,3</sup>**

Item, %	Complex			Simple		
	Fish meal	HP 300	HP 800	Fish meal	HP 300	HP 800
Phase 1 diets						
DM	91.05	91.03	91.14	89.81	89.58	90.00
CP	22.30	22.70	22.90	23.10	23.40	23.30
ADF	3.90	3.90	3.90	4.30	4.10	4.30
NDF	6.80	7.40	7.60	9.00	9.30	8.50
Crude fiber	1.90	2.30	2.20	2.00	2.70	2.60
Ca	0.93	0.84	0.81	0.85	0.89	0.89
P	0.66	0.68	0.68	0.65	0.71	0.67
Cl	0.35	0.25	0.24	0.31	0.25	0.23
Salt	0.57	0.40	0.40	0.51	0.40	0.39
Ether extract	5.10	4.60	4.60	5.20	4.40	4.80
Ash	5.59	5.31	5.30	5.59	5.43	5.61
Starch	21.00	18.40	19.60	26.70	24.40	24.30
Phase 2 diets						
DM	89.96	90.65	89.40	88.72	89.22	89.27
CP	22.60	23.10	23.20	22.80	23.70	23.30
ADF	3.70	4.40	4.30	4.60	4.90	4.20
NDF	8.70	8.60	9.20	10.50	9.00	10.80
Crude fiber	2.50	2.50	2.50	2.60	2.90	2.80
Ca	0.82	0.79	0.80	0.79	0.75	0.72
P	0.60	0.61	0.63	0.59	0.59	0.62
Cl	0.30	0.26	0.24	0.29	0.23	0.24
Salt	0.50	0.42	0.39	0.48	0.39	0.39
Ether extract	5.40	4.80	4.70	5.10	5.30	5.00
Ash	5.21	5.06	5.22	5.03	5.22	5.06
Starch	26.20	24.30	25.40	29.90	29.50	28.10

<sup>1</sup>Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of the diets were then submitted to Ward Laboratories, Inc. (Kearny, NE) for analysis.

<sup>2</sup>Omega Special Select (Omega Protein, Houston, TX).

<sup>3</sup>HP 300 and HP 800 (Hamlet Protein, Findlay, OH).

**Table 5. Laboratory analysis of fishmeal, HP 300, and HP 800<sup>1,2</sup>**

Item, %	Fish meal <sup>3</sup>	HP 300 <sup>4</sup>	HP 800 <sup>4</sup>
DM	91.63 (93.70)	91.67 (92.0)	93.65 (92.00)
CP	63.27 (63.28)	54.50 (56.0)	55.30 (55.00)
ADF	7.10 (0.00)	11.10 (3.7)	10.50 (3.70)
NDF	14.20 (N/A)	13.80 (4.7)	9.00 (4.70)
Crude fiber	0.90 (0.24)	3.90 (3.5)	4.60 (3.50)
Ca	4.62 (4.28)	0.29 (0.25)	0.29 (0.30)
P	2.76 (2.93)	0.71 (0.80)	0.81 (0.80)
Cl	1.23 (N/A)	0.03 (0.06)	0.03 (0.60)
Salt	0.93 (N/A)	0.06 (N/A)	0.04 (N/A)
Ether extract	7.50 (9.71)	1.20 (2.50)	1.40 (2.50)
Ash	18.76 (16.07)	6.06 (6.80)	6.46 (6.50)
Starch	0.20 (0.00)	1.10 (3.80)	1.70 (3.50)

<sup>1</sup>Proximate analysis for proteins sources were analyzed by Ward Laboratories, Kearney, NE.

<sup>2</sup>Values in parenthesis indicate expected analyzed chemical composition values based on the NRC 2012 and Hamlet Protein's nutrient specifications.

<sup>3</sup>Omega Special Select (Omega Protein, Houston, TX).

<sup>4</sup>Hamlet Protein, Findlay, OH.

**Table 6. Effect of diet complexity and specialty protein source on nursery pig performance<sup>1</sup>**

	Complex diet			Simple diet			SEM	Probability, <i>P</i> <		
	Fish meal <sup>2</sup>	HP 300 <sup>3</sup>	HP 800 <sup>3</sup>	Fish meal	HP 300	HP 800		Diet type × protein source	Diet type	Protein source
BW, lb										
d 0	12.9	12.9	12.8	12.9	12.9	12.9	0.35	0.206	0.751	0.369
d 7	13.3	13.4	13.2	13.2	13.2	13.1	0.44	0.968	0.053	0.185
d 21	21.5	21.5	21.1	21.1	21.1	21.0	0.66	0.699	0.158	0.607
d 42	47.2	47.1	47.1	46.5	46.7	47.4	0.71	0.718	0.618	0.832
d 0 to 7										
ADG, lb	0.07	0.07	0.05	0.05	0.06	0.04	0.015	0.923	0.078	0.341
ADFI, lb	0.19	0.19	0.19	0.18	0.19	0.19	0.008	0.741	0.500	0.482
F/G <sup>4</sup>	-	-	-	-	-	-	-	-	-	-
d 7 to 21										
ADG, lb	0.58	0.58	0.56	0.56	0.56	0.57	0.020	0.651	0.293	0.834
ADFI, lb	0.72	0.74	0.73	0.72	0.75	0.73	0.029	0.963	0.920	0.335
F/G	1.24	1.28	1.30	1.29	1.34	1.29	0.028	0.408	0.111	0.216
d 0 to 21										
ADG, lb	0.41	0.41	0.39	0.39	0.39	0.39	0.017	0.734	0.136	0.598
ADFI, lb	0.54	0.56	0.55	0.54	0.56	0.55	0.022	0.897	0.957	0.291
F/G	1.33	1.37	1.40	1.39	1.44	1.41	0.027	0.510	0.037	0.158
d 21 to 42										
ADG, lb	1.22	1.22	1.23	1.21	1.22	1.26	0.048	0.763	0.817	0.517
ADFI, lb	1.72	1.67	1.69	1.66	1.68	1.72	0.041	0.294	0.697	0.548
F/G	1.41	1.37	1.38	1.37	1.38	1.37	0.027	0.305	0.239	0.306
d 0 to 42										
ADG, lb	0.82	0.82	0.81	0.80	0.81	0.82	0.021	0.661	0.674	0.874
ADFI, lb	1.13	1.12	1.12	1.10	1.12	1.13	0.019	0.362	0.742	0.846
F/G	1.39	1.37	1.38	1.38	1.39	1.38	0.021	0.314	0.842	0.916
Economics, \$/pig										
Feed cost	10.20	9.80	9.88	9.34	9.23	9.41	0.161	0.456	0.001	0.289
Feed cost/lb gain <sup>5</sup>	0.30	0.29	0.29	0.28	0.27	0.27	0.004	0.496	0.001	0.002
Total revenue/pig <sup>6,7</sup>	22.30	22.30	22.14	21.82	22.02	22.43	0.562	0.661	0.674	0.874
IOFC <sup>8</sup>	12.09	12.50	12.26	12.48	12.79	13.03	0.433	0.706	0.055	0.395

<sup>1</sup>A total of 720 pigs were used in a 3-phase nursery trial with 10 pigs per pen and 12 replications per treatment. All experimental diets were fed in two phases (d 0 to 7, and d 7 to 21) with a common diet fed in Phase 3 (d 21 to 42).

<sup>2</sup>Omega Special Select Fish meal (Omega Protein, Houston, TX).

<sup>3</sup>HP 300 and HP 800 (Hamlet Protein, Findlay, OH).

<sup>4</sup>Several pens lost weight during Phase 1, thus F/G for this phase is not reported.

<sup>5</sup>Feed cost/lb gain = total feed cost divided by total gain per pig.

<sup>6</sup>One lb of live weight gain was considered to be worth \$0.65.

<sup>7</sup>Total revenue/pig = total gain/pig × \$0.65.

<sup>8</sup>Income over feed cost = total revenue/pig – feed cost/pig.

**Table 7. Main effects of diet complexity on nursery pig performance<sup>1</sup>**

	Complex	Simple	SEM	Probability, $P <$
BW, lb				
d 0	12.9	12.9	0.35	0.751
d 7	13.3	13.2	0.43	0.053
d 21	21.4	21.1	0.62	0.158
d 42	47.2	46.9	0.41	0.618
d 0 to 7				
ADG, lb	0.06	0.05	0.013	0.078
ADFI, lb	0.19	0.18	0.005	0.500
F/G <sup>2</sup>	---	---	---	---
d 7 to 21				
ADG, lb	0.58	0.56	0.015	0.293
ADFI, lb	0.73	0.73	0.025	0.920
F/G	1.27	1.31	0.019	0.111
d 0 to 21				
ADG, lb	0.41	0.39	0.013	0.136
ADFI, lb	0.55	0.55	0.019	0.957
F/G	1.37	1.41	0.016	0.037
d 21 to 42				
ADG	1.22	1.23	0.044	0.817
ADFI	1.70	1.69	0.033	0.697
F/G	1.39	1.37	0.025	0.239
d 0 to 42				
ADG, lb	0.81	0.81	0.016	0.674
ADFI, lb	1.12	1.12	0.011	0.742
F/G	1.38	1.38	0.019	0.842
Economics, \$/pig				
Feed cost	9.96	9.33	0.093	0.001
Feed cost/lb gain <sup>3</sup>	0.29	0.27	0.004	0.001
Total revenue/pig <sup>4,5</sup>	22.24	22.09	0.433	0.674
IOFC <sup>6</sup>	12.28	12.76	0.357	0.055

<sup>1</sup>A total of 720 nursery pigs (PIC C-29 × 359) were used in a 3-phase nursery trial with 10 pigs per pen and 24 replications per treatment for main effects. All experimental diets were fed in two phases (d 0 to 7 and 7 to 21) with a common diet being fed in Phase 3 (d 21 to 42).

<sup>2</sup>Several pens lost weight during Phase 1, thus F/G for this phase is not reported.

<sup>3</sup>Feed cost/lb gain = total feed cost divided by total gain per pig.

<sup>4</sup>One lb of live weight gain was considered to be worth \$0.65.

<sup>5</sup>Total revenue/pig = total gain/pig × \$0.65.

<sup>6</sup>Income over feed cost = total revenue/pig – feed cost/pig.

**Table 8. Main effects of specialty protein source on nursery pig performance<sup>1,2</sup>**

	Fishmeal <sup>3</sup>	HP 300 <sup>4</sup>	HP 800 <sup>4</sup>	SEM	Probability, <i>P</i> <
BW, lb					
d 0	12.9	12.9	12.9	0.35	0.369
d 7	13.3	13.3	13.2	0.43	0.185
d 21	21.3	21.3	21.1	0.63	0.607
d 42	46.8	46.9	47.3	0.50	0.832
d 0 to 7					
ADG, lb	0.06	0.06	0.05	0.014	0.341
ADFI, lb	0.18	0.19	0.19	0.006	0.482
F/G <sup>5</sup>	-	-	-	-	-
d 7 to 21					
ADG, lb	0.57	0.57	0.56	0.016	0.834
ADFI, lb	0.72	0.75	0.73	0.026	0.335
F/G	1.26	1.31	1.30	0.021	0.216
d 0 to 21					
ADG, lb	0.40	0.40	0.39	0.014	0.598
ADFI, lb	0.54	0.56	0.55	0.020	0.291
F/G	1.36	1.40	1.41	0.019	0.158
d 21 to 42					
ADG	1.22	1.22	1.24	0.045	0.517
ADFI	1.69	1.67	1.71	0.035	0.548
F/G	1.39	1.37	1.37	0.026	0.306
d 0 to 42					
ADG, lb	0.81	0.81	0.82	0.017	0.874
ADFI, lb	1.11	1.12	1.13	0.013	0.846
F/G	1.38	1.38	1.38	0.020	0.916
Economics, \$/pig					
Feed cost	9.77	9.52	9.64	0.114	0.289
Feed cost/lb gain <sup>6</sup>	0.29 <sup>a</sup>	0.28 <sup>b</sup>	0.28 <sup>b</sup>	0.004	0.002
Total revenue/pig <sup>7,8</sup>	22.06	22.16	22.28	0.469	0.874
IOFC <sup>9</sup>	12.28	12.64	12.64	0.377	0.395

<sup>1</sup>A total of 720 pigs were used in a 3-phase nursery trial with 10 pigs per pen and 24 replications per treatment for main effects.

<sup>2</sup>All experimental diets were fed in two phases (d 0 to 7, and d 7 to 21) with a common diet being fed in Phase 3 (d 21 to 42).

<sup>3</sup>Omega Special Select Fish meal (Omega Protein, Houston, TX).

<sup>4</sup>HP 300 and HP 800 (Hamlet Protein, Findlay, OH).

<sup>5</sup>Several pens lost weight during Phase 1, thus F/G for this phase is not reported.

<sup>6</sup>Feed cost/lb gain = total feed cost divided by total gain per pig.

<sup>7</sup>One lb of live weight gain was considered to be worth \$0.65.

<sup>8</sup>Total revenue/pig = total gain/pig × \$0.65.

<sup>9</sup>Income over feed cost = total revenue/pig – feed cost/pig.

## Effects of Evosure on Nursery Pig Performance<sup>1,2</sup>

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### Summary

A total of 360 pigs (PIC C-29 × 359, initially 13.1 lb BW) were used in a 42-d growth trial evaluating the effects of Evosure on nursery pig performance. Evosure is a yeast-based technology designed to enhance weaned pig performance. Pigs were weaned at approximately 16 to 20 d and allotted to pens based on initial BW and gender in a completely randomized design. The 3 dietary treatments included a control diet, or the control diet with Evosure (NUTRIQUEST, Inc., Mason City, IA) fed at 1.0 lb/ton fed from d 0 to 21 followed by 0.5 lb/ton fed from d 21 to 42, or 1.0 lb/ton fed from d 0 to 42. Experimental diets were fed in 3 phases (Phase 1, d 0 to 7; Phase 2, d 7 to 21; and Phase 3, d 21 to 42 post-weaning) and in meal form. Overall (d 0 to 42), no differences in growth performance or final BW were observed among dietary treatments. In conclusion, under these experimental conditions, added Evosure, regardless of level, did not impact nursery pig performance.

Key words: Evosure, feed additive, growth performance, nursery pig

### Introduction

Post-weaning pigs undergo physiological and environmental changes that contribute to sub-optimal growth such as low feed intake, body weight loss, and an increase in morbidity and mortality (Pluske, 2013).<sup>4</sup> Some yeast-derived feed additives are suggested to improve gut function and lessen the post-weaning lag that is commonly observed. Evosure is a yeast-based feed additive (NUTRIQUEST, Inc., Mason City, IA) recommended to be included throughout the nursery phase of production. Few published studies are available to determine the optimum level of Evosure to be used at different stages of the nursery period. Therefore, the objective of this study was to determine the influence of Evosure feeding level on growth performance of nursery pigs.

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<sup>2</sup> Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, Inc. for their technical support and expertise in conducting the experiment.

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<sup>4</sup> Pluske, J.R. 2013. Feed and feed additives-related aspects of gut health and development in weanling pigs. J.A.S.B. 4:1. DOI: 10.1186/2049-1891-4-1

## Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. Pens were 5 × 6 ft to allow 3 ft<sup>2</sup> per pig.

A total of 360 pigs (PIC C-29 × 359, initially 13.1 lb BW) with 10 pigs per pen and 12 replications per treatment were used in a 42-d trial. Pigs were weaned at approximately 16 to 20 d of age and allotted to pens based on initial BW and gender in a completely randomized design. Pigs and feeders were weighed every 7 d of the trial to determine ADG, ADFI, and F/G.

The three experimental diets included a control diet, or the control diets with Evosure at 1.0 lb/ton from d 0 to 21 followed by 0.5 lb/ton from d 21 to 42, or 1.0 lb/ton from d 0 to 42. Experimental diets were fed in 3 phases (d 0 to 7, d 7 to 21, and d 21 to 42; Table 1) in meal form. All diets contained an additional 1,897 ppm added Zn from ZnO and 237 ppm added Cu from CuSO<sub>4</sub> above that provided from the trace mineral premix.

Samples of each diet were collected during manufacturing. These samples were submitted for analysis of DM, CP, ether extract, Ca, and P (Ward Laboratories, Inc., Kearney, NE; Table 2).

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Dietary treatment served as the fixed effect in the model. Means are reported as least squares means with individual treatment means used to determine differences. Significant differences between treatments were declared at  $P < 0.05$  and marginal significance defined as  $P < 0.10$ .

## Results and Discussion

Chemical analysis of complete diets revealed that analyzed values were similar to calculated values (Table 2).

There were no differences in growth performance observed among dietary treatments throughout the experimental period (Table 3).

Our results do not agree with previously conducted research where nursery pigs fed Evosure had improved growth performance. Previous research conducted by Nutriquest showed that the addition of Evosure to weaned pig starter diets improved ADG and F/G by 10.9% and 5.2%, respectively.<sup>5</sup> Additional research should be conducted to determine the optimum Evosure feeding level and duration to maximize performance and to determine if different basal diet formulations or other factors might influence the responses observed.

<sup>5</sup> Nutriquest Launches Product for Starter Pigs. NUTRIQUEST. <http://www.farms.com/news/nutriquest-launches-product-for-starter-pigs-70961.aspx>. 2013.

**Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	32.10	47.82	62.05
Soybean meal, 48% CP	27.50	28.93	32.23
Spray dried whey	20.0	14.50	---
Fish meal	3.50	3.25	---
Cheese plus <sup>2</sup>	7.65	---	---
Crystalline lactose	3.55	---	---
betaGRO <sup>3</sup>	0.27	---	---
Evosure <sup>3,4</sup>	---	---	---
Tallow	2.00	2.00	2.00
Limestone	0.85	1.05	1.1
Monocalcium P, 21% P	1.05	0.95	0.85
Sodium chloride	0.25	0.25	0.5
L-Lys HCl	0.25	0.3	0.35
DL-Met	0.21	0.17	0.15
L-Thr	0.14	0.11	0.12
L-Trp	0.01	---	---
Phytase	---	---	0.01
Zinc oxide	0.26	0.26	0.26
Copper sulfate	0.09	0.09	0.09
Choline chloride, 70% liquid	0.05	0.05	0.05
Selenium, 0.06%	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09
Vitamin premix	0.05	0.05	0.05
Vitamin E, 20,000 IU/lb	0.06	0.06	0.06
Biotin	0.08	0.08	0.08
Total	100	100	100

*continued*

Calculated analysis

Standardized ileal digestible (SID) amino acids, %

Lys	1.52	1.35	1.25
Met:Lys	38	36	35.0
Met & Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	18	18	18
Val:Lys	65	67	68
Total Lysine, %	1.68	1.50	1.39
ME, kcal/lb	1,588	1,538	1,533
CP, %	23.3	21.7	20.7
Ca, %	0.90	0.90	0.69
P, %	0.78	0.72	0.71
Available P, %	0.55	0.45	0.40
Cu, ppm	265	263	258
Zn, ppm	2,023	2,025	2,025

<sup>1</sup>Phase 1 diets were fed from d 0 to 7 (~13.1 to 14 lb BW), Phase 2 diets from d 7 to 21 (~14 to 24 lb BW) and Phase 3 diets from d 21 to 42 (~24 to 51 lb BW).

<sup>2</sup>International Ingredients, Inc., St. Louis, MO.

<sup>3</sup>NUTRIQUEST, Inc., Mason City, IA.

<sup>4</sup>Treatment diets included: 1.) No Evosure fed from d 0 to 42, 2.) 1 lb/ton Evosure fed from d 0 to 21 followed by 0.5 lb/ton Evosure from d 21 to 42, and 3.) 1 lb/ton Evosure fed from d 0 to 42.

<sup>5</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of diet, with a release of 0.13% available P.

**Table 2. Chemical analysis of experimental diets<sup>1</sup>**

Item, %	Control	Evosure lb/ton,	
		1/0.5 <sup>2</sup>	1/1 <sup>3</sup>
Phase 1 Diets			
DM	90.8	90.5	90.5
CP	23.2	24.5	24.5
Ether extract	5.70	5.10	5.10
Ca	1.07	1.07	1.07
P	0.82	0.80	0.80
Phase 2 diets			
DM	89.8	89.5	89.5
CP	21.0	22.3	22.3
Ether extract	4.60	4.20	4.20
Ca	1.22	0.99	0.99
P	0.82	0.73	0.73
Phase 3 diets			
DM	88.0	87.4	87.2
CP	21.9	19.3	20.1
Ether extract	5.00	4.60	4.80
Ca	0.98	0.82	0.82
P	0.61	0.56	0.58

<sup>1</sup>Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of diets were then analyzed for DM, CP, ether extract, Ca, and P (Ward Laboratories, Inc., Kearney, NE).

<sup>2</sup>Evosure (NUTRIQUEST, Inc., Mason City, IA) fed at 1 lb/ton from d 0 to 21 followed by 0.5 lb/ton from d 21 to 42.

<sup>3</sup>Evosure fed at 1 lb/ton from d 0 to 42.

**Table 3. Effects of Evosure on growth performance of nursery pigs <sup>1</sup>**

Item	Control	Evosure, lb/ton		SEM	<i>P</i> <
		1/0.5 <sup>2</sup>	1/1 <sup>3</sup>		
<b>BW, lb</b>					
d 0	13.69	13.71	13.72	0.019	0.466
d 7	14.46	14.35	14.51	0.076	0.331
d 21	25.77	25.51	25.78	0.247	0.680
d 42	56.19	56.07	56.21	0.549	0.980
<b>d 0 to 21</b>					
ADG, lb	0.57	0.56	0.57	0.011	0.728
ADFI, lb	0.72	0.69	0.72	0.011	0.163
F/G	1.26	1.24	1.26	0.015	0.489
<b>d 21 to 42</b>					
ADG, lb	1.45	1.45	1.45	0.019	0.986
ADFI, lb	2.18	2.17	2.18	0.023	0.954
F/G	1.51	1.50	1.50	0.018	0.861
<b>d 0 to 42</b>					
ADG, lb	1.00	1.00	1.01	0.013	0.905
ADFI, lb	1.44	1.43	1.45	0.015	0.595
F/G	1.44	1.43	1.43	0.013	0.741

<sup>1</sup>A total of 360 pigs (PIC C-29 × 359) were used in a 3-phase nursery trial with 10 pigs per pen and 12 replications per treatment. All experimental diets were fed in three phases (d 0 to 7, 7 to 21, and 21 to 42).

<sup>2</sup>Evosure (NUTRIQUEST, Inc., Mason City, IA) fed at 1 lb/ton from d 0 to 21 followed by 0.5 lb/ton from d 21 to 42.

<sup>3</sup>Evosure fed at 1 lb/ton from d 0 to 42.

## Effects of a Gluco-oligosaccharide on Growth Performance of Nursery Pigs<sup>1</sup>

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### Summary

A total of 3,456 nursery pigs (PIC L337 × 1050, initially 12.4 lb BW) were housed in 3 commercial research rooms and used in a 42-d growth study to determine the effects of gluco-oligosaccharide (Midori USA, Inc., Cambridge, MA) on growth performance. In each room, pens of pigs (27 pigs/pen) were blocked (6, 5, and 5 blocks in rooms 1, 2, and 3, respectively) by initial pen BW. Within blocks, pens were allotted randomly to 1 of 8 dietary treatments in a 2-phase feeding program (d 0 to 14 and d 14 to 42). Dietary treatments were arranged in a 2 × 3 factorial, with or without antibiotic (0 or 55 ppm, Carbadox, Phibro Pro, Teaneck, NJ) and 4 concentrations of gluco-oligosaccharide (0, 200, 400, and 600 ppm). Gluco-oligosaccharide product used in rooms 1 and 2 originated from a different batch than that used in room 3. For the overall feeding period, no room × antibiotic × gluco-oligosaccharide or antibiotic × gluco-oligosaccharide interactions were observed for any growth responses, but tendencies were found ( $P < 0.10$ ) for room × gluco-oligosaccharide interaction for final BW and ADG. In rooms 1 and 2, antibiotic treatment increased ADG and ADFI in all feeding periods and improved F/G from d 14 to 28 and d 28 to 42. Increasing gluco-oligosaccharide improved (linear,  $P < 0.05$ ) ADG and F/G from d 0 to 14. It also increased ( $P = 0.047$ ) ADG and tended ( $P = 0.087$ ) to increase ADFI from d 14 to 28, but did not alter the growth responses from d 28 to 42. For the overall period (d 0 to 42), adding an antibiotic to the diet increased ( $P < 0.01$ ) ADG and ADFI, but did not affect F/G. Increasing gluco-oligosaccharide improved (linear,  $P < 0.01$ ) ADG and F/G and tended ( $P = 0.063$ ) to linearly increase ADFI. In room 3, a much smaller response was observed for antibiotic inclusion with only improved ( $P = 0.005$ ) F/G from d 14 to 28 and increased ( $P < 0.05$ ) ADG and ADFI from d 28 to 42. Pigs fed increasing gluco-oligosaccharide tended (linear,  $P < 0.10$ ) to have reduced ADG and ADFI; however, the overall growth performance was not affected by antibiotic or gluco-oligosaccharide treatments. In conclusion, feeding gluco-oligosaccharide may improve growth performance in nursery pigs, and this effect appears to be independent of the use of antibiotic and more prominent during the early nursery phase. However, due to some room × gluco-oligosaccharide interactions, further research is required to confirm the consistency of the responses to the gluco-oligosaccharide used in this study.

<sup>1</sup> Appreciation is expressed to Midori USA, Inc. (Cambridge, MA) for partial funding and New Horizon Farms (Pipestone, MN) for providing the animals, research facilities, and technical support.

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Key words: antibiotic, gluco-oligosaccharide, growth, nursery pig

## Introduction

Oligosaccharides are a group of carbohydrate polymers containing 3 to 10 simple sugars that can be fed to pigs as prebiotics. Mannan- (Davis et al., 2002;<sup>3</sup> Rozeboom et al., 2005<sup>4</sup>), chito- (Liu et al., 2008<sup>5</sup>), and fructo-oligosaccharides (Gebbinck et al., 1999<sup>6</sup>), have been shown to improve growth performance in young pigs. Possible mechanisms by which oligosaccharides benefit growth performance have been proposed and center on improving health status of the pig. For example, oligosaccharide may interact with intestinal mucosa and prevent pathogens, e.g., *E. coli* and *Salmonella*, from colonizing and proliferating at the mucosal surface (Miguel et al., 2004<sup>7</sup>). Oligosaccharide may also enhance the immune system of pigs by increasing antibody titers, immunoglobulins, and macrophage activities (Davis et al., 2004<sup>8</sup>). In addition, antibiotics have been widely fed to nursery pigs as growth promoters; however, concerns with antibiotic resistance have led to a ban on the use of growth promoting antibiotics that are medically important for human use (FDA, 2015<sup>9</sup>) in swine diets. Therefore, oligosaccharide products have been proposed as the alternatives to antibiotics in nursery pig diets. The objective of this study was to determine the effects of feeding a gluco-oligosaccharide with or without a feed grade antibiotic on growth performance of nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care Committee approved the protocol used in the experiment. The study was conducted at a commercial nursery research facility in southwest Minnesota. The barn was mechanically ventilated and temperature was maintained at approximately 80°F. Each pen (12.1 × 7.5 ft<sup>2</sup>) had completely slatted plastic floors and was equipped with a 6-hole, stainless-steel, dry self-feeder and a pan waterer. Pigs were allowed ad libitum access to feed and water throughout the experiment. Diets were manufactured at a local feed mill (New Horizon Farms, Pipestone, MN). Feed additions to each individual pen were delivered and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

<sup>3</sup> Davis, M. E., C. V. Maxwell, D. C. Brown, B. Z. De Rodas, Z. B. Johnson, E. B. Kegley, D. H. Hellwig, and R. A. Dvorak. 2002. Effect of dietary mannan oligosaccharides and(or) pharmacological additions of copper sulfate on growth performance and immunocompetence of weanling and growing/finishing pigs. *J. Anim. Sci.* 80:2887–2894.

<sup>4</sup> Rozeboom, D. W., D. T. Shaw, R. J. Tempelman, J. C. Miguel, J. E. Pettigrew and A. Connolly. 2005. Effect of mannan oligosaccharide and an antimicrobial product in nursery diets on performance of pigs reared on three different farms. *J. Anim. Sci.* 83:2637-2644.

<sup>5</sup> Liu, P., X. S. Piao, S. W. Kim, L. Wang, Y. B. Shen, H. S. Lee, and S. Y. Li. 2008. Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and *Lactobacillus* in weaning pigs. *J. Anim. Sci.* 86:2609-2618.

<sup>6</sup> Gebbinck, G. A. R., A. L. Sutton, B. T. Richert, J. A. Patterson, J. Nielsen, D. T. Kelly, M. W. A. Verstegen, B. A. Williams, M. Bosch, M. Cobb, D. C. Kendall, S. DeCamp, and K. Bowers. 1999. Effects of addition of fructooligosaccharide (FOS) and sugar beet pulp to weanling pig diets on performance, microflora and intestinal health. *Swine Day*, vol. 31. Purdue University, pp. 53–59.

<sup>7</sup> Miguel, J. C., S. L. Rodriguez-Zas, and J. E. Pettigrew. 2004. Efficacy of a mannan oligosaccharide (BioMos<sup>®</sup>) for improving nursery pig performance. *J. Swine Health Prod.* 12:296–307.

<sup>8</sup> Davis, M. E., D. C. Brown, C. V. Maxwell, Z. B. Johnson, E. B. Kegley, and R. A. Dvorak. 2004. Effect of phosphorylated mannans and pharmacological additions of zinc oxide on growth and immunocompetence of weanling pigs. *J. Anim. Sci.* 82:581–587.

<sup>9</sup> FDA. 2015. Federal register. 80: No. 106.

This experiment was replicated twice. In replicate 1, pigs ( $n = 2,376$ ; initial BW = 11.9 lb; PIC L337  $\times$  1050) were housed in two rooms (48 pens in room 1 and 40 pens in room 2). Replicate 2 was conducted with the next group of pigs placed into room 1, but will be referred to as room 3 for ease of clarification. In room 3, pigs ( $n = 1,080$ ; initial BW = 13.5 lb; PIC L337  $\times$  1050) were housed in 40 pens. In each room, pens of pigs (27 pigs/pen) were blocked (6, 5, and 5 blocks in rooms 1, 2, and 3, respectively) by initial pen BW and allotted randomly to 1 of 8 dietary treatments. The dietary treatments were arranged in a  $2 \times 3$  factorial, with or without antibiotic (0 or 55 ppm Carbadox, Phibro Animal Health Corp., Teaneck, NJ), and 4 levels of gluco-oligosaccharide (0, 200, 400, and 600 ppm; Midori USA, Inc., Cambridge, MA). The basal diets used in the study are provided in Table 1. Antibiotic and/or gluco-oligosaccharide were added to the basal diets at the expense of corn. The 0 and 600 ppm gluco-oligosaccharide diets were manufactured and used to blend in the robotic feeding system to provide diets with 200 and 400 ppm gluco-oligosaccharide (Table 2). Gluco-oligosaccharide product used in rooms 1 and 2 originated from a different batch from that of product used in room 3. Pigs were fed in 2 phases from d 0 to 14 and d 14 to 42. Pens were weighed and feed disappearance was measured every 7 d to determine ADG, ADFI, and F/G. Diet samples were taken from six feeders per dietary treatment, delivered to Kansas State University Swine Laboratory, and stored at  $-20^{\circ}\text{C}$ . Diet samples were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, crude fat, Ca, and P. Diet samples were also sent to Phibro Animal Health Corp. Feed Laboratory (State College, PA) for the analysis of Carbadox concentrations.

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The statistical model included fixed effects of room, antibiotic, gluco-oligosaccharide, and their interactions, with block as a random effect. The statistical model was simplified by removing the room  $\times$  antibiotic  $\times$  gluco-oligosaccharide interaction ( $P > 0.10$ ), and the degrees of freedom of non-significant interactions were pooled to test the remaining fixed effects. Linear and quadratic contrasts were conducted among the gluco-oligosaccharide concentrations and a single degree of freedom contrast was used to compare the treatments with and without antibiotic. Results were considered significant at  $P < 0.05$  and a tendency at  $0.05 < P < 0.10$ .

## Results and Discussion

Analyzed chemical composition of dietary treatments generally matched formulated nutrient levels. Analyzed CP and antibiotic concentrations were slightly lower than the formulated levels, but were consistent across treatments, phases, and rooms.

The  $P$  values for the fixed effects on ADG, ADFI, F/G, and BW are shown in Table 3. No room  $\times$  antibiotic  $\times$  gluco-oligosaccharide interactions were significant ( $P > 0.10$ ) for any of the growth responses and, therefore, were removed from the statistical model. Tendencies for room  $\times$  gluco-oligosaccharide interactions were observed for final BW ( $P = 0.059$ ; Figure 1) and overall ADG ( $P = 0.087$ ; Figure 2). Pigs from rooms 1 and 2 had similar response trends to the gluco-oligosaccharide but shared different patterns than that of pigs in room 3 (Figure 1 and 2). Pigs from the first (room 1 and 2) and second (room 3) replicates of the experiment were from different batches, raised in different time points, and fed a different batch of the gluco-oligosaccharide, which might

explain the discrepancy in pig performance between experimental replicates. Therefore, data from room 1 and 2 were pooled to test the treatment effects of gluco-oligosaccharide and antibiotic separately from room 3.

No interactive effects among room, antibiotic, and gluco-oligosaccharide were observed for removal rate ( $P > 0.42$ ). Percentage of pigs removed from the experiment was not affected by the antibiotic or gluco-oligosaccharide treatments, but tended ( $P = 0.064$ ) to vary among rooms. Removal rate in room 3 (4.2%) was greater ( $P < 0.05$ ) than in room 2 (1.9%), but was not statistically different from that in room 1 (2.8%); no differences were observed between removal rates in room 1 and 2.

No antibiotic  $\times$  gluco-oligosaccharide interactions were observed in the analyses of growth responses. In a review of 29 studies, Miguel et al. (2004<sup>7</sup>) concluded that the effects of feeding mannan-oligosaccharide on growth performance of nursery pigs were independent to the application of an antibiotic in the diet, and the effects are additive. Growth performance of pigs fed in rooms 1 and 2 is presented in Table 4. Body weight of pigs fed antibiotic was greater ( $P = 0.073$ ) at d 14 and ( $P < 0.01$ ) at d 28 and 42. Feeding an antibiotic improved ( $P = 0.026$ ) ADG, tended to increase ( $P = 0.067$ ) ADFI, but did not affect F/G of pigs from d 0 to 14. Pigs fed diets containing antibiotic had improved ( $P < 0.05$ ) ADG, ADFI, and F/G compared with those fed diets without antibiotic from d 14 to 28 and 28 to 42. For the overall feeding period (d 0 to 42), ADG and ADFI were improved ( $P < 0.01$ ), but F/G was unaffected by addition of a dietary antibiotic.

In rooms 1 and 2, increasing gluco-oligosaccharide increased (linear,  $P < 0.01$ ) BW on d 14, 28, and 42. Increasing gluco-oligosaccharide improved (linear,  $P < 0.01$ ) ADG and F/G, but did not affect ADFI from d 0 to 14. From d 14 to 28, increasing gluco-oligosaccharide increased (linear,  $P = 0.047$ ) ADG and tended to increase (linear,  $P = 0.087$ ) ADFI, but had no effect on F/G. Growth performance of pigs from d 28 to 42 were not affected by added gluco-oligosaccharide. For the overall period (d 0 to 42), increasing gluco-oligosaccharide improved (linear,  $P < 0.01$ ) ADG and F/G and tended to increase (linear,  $P = 0.063$ ) ADFI. Improved pig growth performance during nursery phases has been reported in other studies (Davis et al., 2002;<sup>3</sup> Rozeboom et al., 2005;<sup>4</sup> Liu et al., 2008<sup>5</sup>) when mannan- or chito-oligosaccharides were added in the diets. Miguel et al. (2004<sup>7</sup>) suggested that pigs in the first 1 to 2 weeks post-weaning with relatively slow growth rate had more prominent response to oligosaccharide products than older nursery pigs, which supported our findings that gluco-oligosaccharide treatment promoted ADG and F/G during d 0 to 14 and increased ADG and ADFI from d 14 to 28 but did not affect growth responses from d 28 to 42.

Growth performance of pigs in room 3 is presented in Table 5. Neither the antibiotic nor gluco-oligosaccharide treatments affected the BW of pigs. In contrast to the observations in rooms 1 and 2, a much smaller response was observed for dietary antibiotic addition in room 3, with the only improved ( $P = 0.005$ ) F/G from d 14 to 28 and increased ( $P < 0.05$ ) ADG and ADFI from d 28 to 42. No response was observed for added gluco-oligosaccharide, except that pigs tended (linear,  $P < 0.10$ ) to have decreased ADG and ADFI from d 14 to 28 with increasing gluco-oligosaccharides. Discrepancies in pigs' responses to gluco-oligosaccharide treatment between experimen-

tal replicates might be attributed to the environment, health status of pigs, quality of dietary ingredients, as well as many other factors (Miguel et al., 2004<sup>7</sup>). Rozeboom et al. (2005<sup>4</sup>) also reported inconsistent responses of pigs to dietary mannan-oligosaccharide in an experiment where improved ADG, ADFI, and F/G were observed in one research farm, but these responses were not able to be replicated in another two farms during the same feeding period; likewise, antibiotics were reported to enhance pig growth performance in two out of the three farms, but no effect was observed in the third farm.

In summary, these results suggest that the gluco-oligosaccharide used in these studies may improve growth performance of nursery pigs, especially during the early post-weaning period, and the magnitude of these effects may be related to the concentration of gluco-oligosaccharide and independent to the use of antibiotic in the diets. However, further research is required to confirm the consistency of pigs' responses to antibiotic and gluco-oligosaccharide treatments.

**Table 1. Composition of base diets (as-fed basis)**

Items	Phase 1 <sup>1</sup>	Phase 2 <sup>1</sup>
Ingredients, %		
Corn	50.53	56.85
Soybean meal (48% CP)	25.35	29.81
Corn DDGS, 6-9% oil	7.50	10.00
Fish meal	3.75	0.00
Dried whey	10.00	0.00
Calcium carbonate	0.90	1.15
Monocalcium phosphate (22% P)	0.35	0.80
Sodium chloride	0.35	0.35
L-Lys HCl	0.40	0.45
DL-Met	0.15	0.13
L-Thr	0.16	0.15
L-Trp	0.03	0.02
Phytase <sup>2</sup>	0.03	0.03
Zinc oxide	0.26	0.00
Tri-basic copper chloride	0.03	0.03
Trace mineral premix	0.13	0.13
Vitamin premix	0.10	0.10
Antibiotic <sup>3</sup>	---	---
Gluko-oligosaccharide premix <sup>4</sup>	---	---
Total	100.00	100.00

*continued*

**Table 1. Composition of base diets (as-fed basis)**

Items	Phase 1 <sup>1</sup>	Phase 2 <sup>1</sup>
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	1.35	1.30
Ile:Lys	59	60
Leu:Lys	125	131
Met:Lys	36	34
Met and Cys:Lys	57	56
Thr:Lys	64	63
Trp:Lys	18	19
Val:Lys	65	66
Total Lys, %	1.52	1.47
CP, %	22.44	22.28
ME, kcal/lb	1,502	1,490
NE, kcal/lb	1,015	966
SID Lys:ME, g/Mcal	4.08	3.96
Ca, %	0.73	0.70
P, %	0.61	0.59
Available P, %	0.47	0.42

<sup>1</sup> Phase 1 diets were fed from d 0 to 14, and Phase 2 diets were fed from d 14 to 42.

<sup>2</sup> Optiphos 2000 (Enzyvia, Sheridan, IN).

<sup>3</sup> Carbadox (Mecadox, Phibro Animal Health Corp., Teaneck, NJ); product was added to the base diets at 55 ppm to form antibiotic treatments.

<sup>4</sup> Midori USA, Inc. (Cambridge, MA); product was added to the base diets at either 200, 400, or 600 ppm to form gluco-oligosaccharide treatments.

**Table 2. Analyzed composition of experimental diets (as-fed basis)<sup>1</sup>**

	Phase 1 <sup>2</sup>								Phase 2 <sup>2</sup>								
	Antibiotic, <sup>3</sup> ppm:	0	0	0	0	55	55	55	55	0	0	0	0	55	55	55	55
	Glucosaccharide, <sup>4</sup> ppm:	0	200	400	600	0	200	400	600	0	200	400	600	0	200	400	600
Room 1 and 2																	
DM, %		89.4	89.0	89.8	89.4	89.0	89.1	89.6	89.4	88.7	88.9	88.3	88.2	89.0	88.4	88.9	88.5
CP, %		20.2	20.5	21.2	21.4	20.3	21.1	20.7	21.7	19.8	21.0	18.8	19.2	19.7	20.5	20.6	21.4
Fat, %		3.0	2.8	2.8	2.8	2.8	2.7	2.7	2.8	3.3	3.0	2.9	2.9	2.8	2.9	3.1	2.9
Ca, %		0.70	0.81	0.84	0.77	0.90	1.00	1.00	1.02	0.81	0.72	0.65	0.77	0.99	0.88	0.83	0.96
P, %		0.61	0.62	0.60	0.62	0.58	0.63	0.64	0.68	0.59	0.60	0.58	0.62	0.61	0.64	0.63	0.63
Carbadox, <sup>5</sup> ppm		< 1	---	---	< 1	47.0	---	---	45.0	< 1	---	---	1.5	50.0	---	---	41.0
Room 3																	
DM, %		89.1	88.4	88.9	88.9	88.5	88.4	88.4	90.1	87.5	87.0	86.9	86.9	87.4	86.9	87.2	86.9
CP, %		20.2	20.7	21.5	21.7	21.2	21.4	21.6	22.2	21.9	19.5	21.6	21.3	20.4	21.1	21.9	21.7
Fat, %		3.1	3.1	3.0	2.9	2.9	3.0	2.8	2.9	2.9	2.7	2.8	2.7	2.5	2.6	2.6	3.2
Ca, %		0.77	0.84	0.80	0.79	0.91	0.94	1.05	0.95	0.74	0.75	0.76	0.80	1.16	1.06	0.97	0.85
P, %		0.57	0.60	0.61	0.63	0.62	0.65	0.64	0.64	0.57	0.60	0.63	0.60	0.59	0.62	0.60	0.60
Carbadox, <sup>5</sup> ppm		< 1	---	---	< 1	41.0	---	---	51.0	< 1	---	---	< 1	42.0	---	---	45.0

<sup>1</sup> Multiple samples of each diet were collected, blended and subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE).

<sup>2</sup> Phase 1 diets were fed from d 0 to 14, and Phase 2 diets were fed from d 14 to 42.

<sup>3</sup> Carbadox (Mecadox, Phibro Animal Health Corp., Teaneck, NJ).

<sup>4</sup> Gluco-oligosaccharide (Midori USA, Inc., Cambridge, MA).

<sup>5</sup> The diets with lowest and highest oligosaccharide content were tested for Carbadox as they were blended for the intermediate treatments.

**Table 3. *P* values for the sources of variation in the analyses of growth performance<sup>1</sup>**

Source of variation	d 0 to 14	d 14 to 28	d 28 to 42	d 0 to 42
BW <sup>2,3</sup>				
Antibiotic <sup>4</sup>	0.472	<0.001	<0.001	---
Gluko-oligosaccharide <sup>5</sup>	0.008	0.150	0.195	---
Room	<0.001	0.030	0.188	---
Antibiotic × gluco-oligosaccharide	0.410	0.505	0.603	---
Room × antibiotic	0.143	0.063	0.220	---
Room × gluco-oligosaccharide	0.772	0.168	0.059	---
ADG <sup>3</sup>				
Antibiotic	0.294	<0.001	<0.001	<0.001
Gluko-oligosaccharide	0.006	0.897	0.842	0.304
Room	<0.001	<0.001	0.093	0.002
Antibiotic × gluco-oligosaccharide	0.612	0.417	0.139	0.446
Room × antibiotic	0.083	0.143	0.947	0.243
Room × gluco-oligosaccharide	0.803	0.162	0.033	0.087
ADFI <sup>3</sup>				
Antibiotic	0.308	0.044	<0.001	<0.001
Gluko-oligosaccharide	0.397	0.761	0.591	0.559
Room	0.001	<0.001	0.235	0.065
Antibiotic × gluco-oligosaccharide	0.433	0.523	0.327	0.524
Room × antibiotic	0.244	0.139	0.490	0.201
Room × gluco-oligosaccharide	0.993	0.234	0.188	0.409
F/G <sup>3</sup>				
Antibiotic	0.958	<0.001	0.078	0.054
Gluko-oligosaccharide	0.007	0.724	0.883	0.070
Room	<0.001	0.026	0.666	0.033
Antibiotic × gluco-oligosaccharide	0.937	0.202	0.521	0.486
Room × antibiotic	0.234	0.997	0.085	0.609
Room × gluco-oligosaccharide	0.534	0.744	0.338	0.259

<sup>1</sup> A total of 3,456 pigs (PIC L337 × 1050, initially 12.4 lb BW) were used in a 42-d study. Pigs were housed in 3 commercial research rooms with 27 pigs per pen and a total of 16 pens per treatment.

<sup>2</sup> Body weight of pigs was recorded at the end of a feeding period.

<sup>3</sup> Effects of room × antibiotic × oligosaccharide interaction were not significant ( $P > 0.40$ ) for the overall trial (d 0 to 42) and therefore, were removed from the statistical model.

<sup>4</sup> Carbadox (Mecadox, Phibro Animal Health Corp., Teaneck, NJ).

<sup>5</sup> Gluko-oligosaccharide (Midori USA, Inc., Cambridge, MA).

**Table 4. Effects of antibiotic and increasing gluco-oligosaccharide on growth performance of pigs (Rooms 1 and 2)<sup>1</sup>**

Item	Antibiotic, <sup>2</sup> ppm		Gluco-oligosaccharide, <sup>3</sup> ppm					Probability, <i>P</i> <			
								Gluco-oligosaccharide			
	0	55	SEM	0	200	400	600	SEM	Antibiotic	Linear	Quadratic
Removal, %	2.54	2.16	0.005	3.03	1.32	2.73	2.75	0.008	0.564	0.704	0.161
BW, lb											
d 0	11.9	11.9	0.12	11.8	11.9	11.9	12.0	0.13	0.596	0.115	0.712
d 14	21.1	21.3	0.23	20.8	21.1	21.4	21.5	0.25	0.073	0.001	0.758
d 28	35.0	36.3	0.41	35.0	35.5	35.9	36.2	0.45	<0.001	0.001	0.532
d 42	52.1	54.0	0.46	52.1	52.9	53.2	53.9	0.54	<0.001	0.002	0.985
d 0 to 14											
ADG, lb	0.58	0.60	0.008	0.56	0.58	0.59	0.61	0.010	0.026	0.000	0.901
ADFI, lb	0.79	0.81	0.010	0.79	0.80	0.81	0.80	0.012	0.067	0.224	0.475
F/G	1.37	1.36	0.019	1.41	1.38	1.36	1.32	0.022	0.305	<0.001	0.652
d 14 to 28											
ADG, lb	0.99	1.06	0.015	1.00	1.03	1.03	1.04	0.018	<0.001	0.047	0.403
ADFI, lb	1.41	1.45	0.019	1.40	1.43	1.45	1.44	0.022	0.010	0.087	0.317
F/G	1.42	1.38	0.007	1.40	1.39	1.40	1.39	0.010	<0.001	0.502	0.911
d 28 to 42											
ADG, lb	1.20	1.26	0.012	1.21	1.24	1.22	1.24	0.016	0.001	0.230	0.958
ADFI, lb	1.86	1.97	0.016	1.90	1.91	1.92	1.94	0.022	<0.001	0.146	0.785
F/G	1.55	1.57	0.016	1.56	1.55	1.57	1.56	0.018	0.020	0.712	0.710
d 0 to 42											
ADG, lb	0.92	0.97	0.008	0.92	0.95	0.95	0.96	0.010	<0.001	0.004	0.610
ADFI, lb	1.35	1.41	0.013	1.36	1.38	1.39	1.39	0.016	<0.001	0.063	0.550
F/G	1.46	1.46	0.008	1.47	1.46	1.46	1.45	0.009	0.136	0.007	0.998

<sup>1</sup> A total of 2,376 pigs (PIC L337 × 1050, initially 11.9 lb BW) were housed in rooms 1 and 2 and used in a 42-d study. Room 1 contained 48 pens with 6 pens per treatment and room 2 contained 40 pens with 5 pens per treatment.

<sup>2</sup> Carbadox (Mecadox, Phibro Animal Health Corp., Teaneck, NJ).

<sup>3</sup> Midori USA, Inc. (Cambridge, MA).

**Table 5. Effects of antibiotic and increasing gluco-oligosaccharide on growth performance of pigs (Room 3)<sup>1</sup>**

Item	Antibiotic, <sup>2</sup> ppm			Gluco-oligosaccharide, <sup>3</sup> ppm					Probability, <i>P</i> <		
	0	55	SEM	0	200	400	600	SEM	Antibiotic	Gluco-oligosaccharide	
										Linear	Quadratic
Removal, %	4.09	4.22	0.011	3.05	5.49	4.64	3.84	0.016	0.924	0.729	0.231
BW, lb											
d 0	13.5	13.5	0.18	13.4	13.4	13.5	13.6	0.19	0.561	0.079	0.541
d 14	23.2	22.9	0.34	22.8	23.1	23.2	23.1	0.37	0.237	0.333	0.399
d 28	34.4	34.6	0.61	34.6	34.7	34.5	34.1	0.67	0.691	0.370	0.604
d 42	53.4	54.1	0.68	54.1	53.5	54.5	53.0	0.81	0.299	0.389	0.414
d 0 to 14											
ADG, lb	0.54	0.53	0.012	0.53	0.53	0.54	0.54	0.016	0.224	0.427	0.941
ADFI, lb	0.85	0.84	0.015	0.83	0.85	0.84	0.85	0.018	0.461	0.450	0.549
F/G	1.57	1.60	0.028	1.58	1.60	1.59	1.57	0.033	0.208	0.701	0.498
d 14 to 28											
ADG, lb	0.86	0.89	0.023	0.90	0.87	0.87	0.85	0.027	0.250	0.070	0.856
ADFI, lb	1.21	1.20	0.028	1.24	1.21	1.21	1.17	0.034	0.920	0.073	0.777
F/G	1.40	1.36	0.011	1.37	1.39	1.39	1.38	0.015	0.005	0.856	0.322
d 28 to 42											
ADG, lb	1.23	1.27	0.018	1.27	1.22	1.29	1.22	0.024	0.029	0.505	0.707
ADFI, lb	1.89	1.96	0.025	1.95	1.89	1.98	1.88	0.034	0.035	0.495	0.509
F/G	1.54	1.54	0.024	1.54	1.55	1.54	1.54	0.026	0.865	0.926	0.530
d 0 to 42											
ADG, lb	0.88	0.90	0.012	0.90	0.88	0.90	0.88	0.016	0.214	0.396	0.997
ADFI, lb	1.33	1.35	0.020	1.35	1.32	1.36	1.31	0.024	0.350	0.391	0.615
F/G	1.50	1.49	0.011	1.49	1.50	1.50	1.49	0.013	0.215	0.917	0.127

<sup>1</sup> A total of 1,080 pigs (PIC L337 × 1050, initially 13.5 lb BW) were housed in rooms 3 and used in a 42-d study. Room 3 contained 40 pens with 5 pens per treatment.

<sup>2</sup> Carbadox (Mecadox, Phibro Pro, Teaneck, NJ).

<sup>3</sup> Midori USA, Inc. (Cambridge, MA).

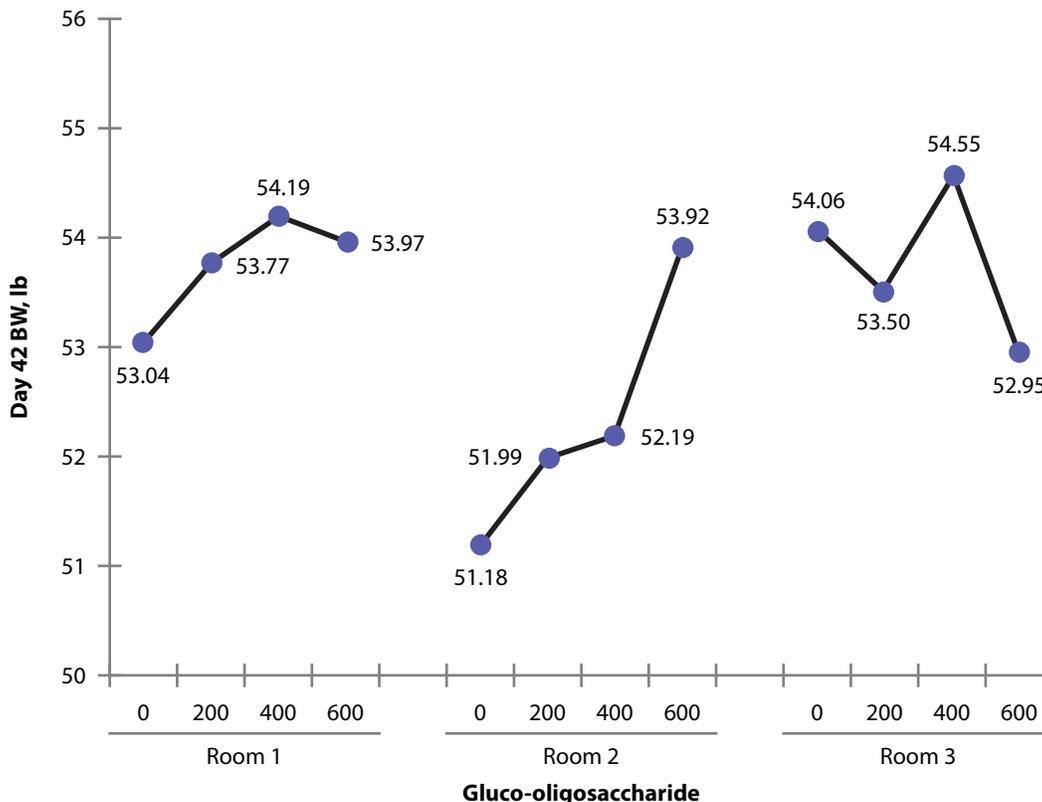


Figure 1. Effects of room × gluco-oligosaccharide interaction on day 42 BW ( $P = 0.059$ ).

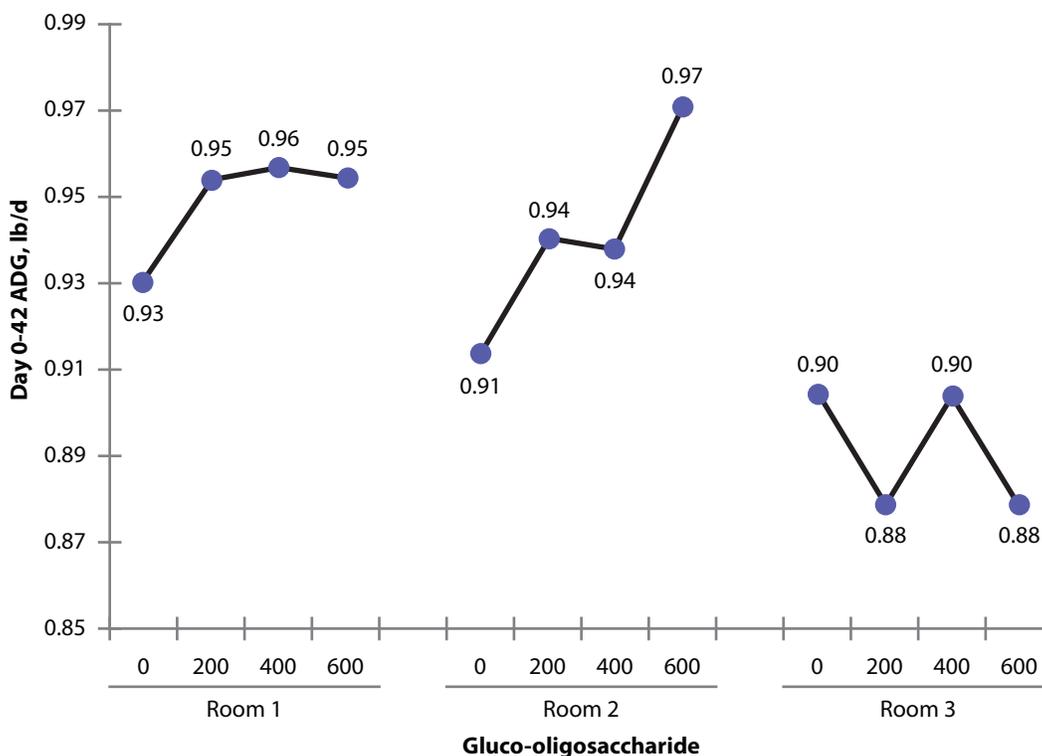


Figure 2. Effects of room × gluco-oligosaccharide interaction on overall ADG ( $P = 0.087$ ).

## Evaluation of Elarom SES in Nursery Diets with or without the Inclusion of High Zinc Oxide or Feed Antimicrobials<sup>1</sup>

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### Summary

A total of 360 weaned pigs (DNA 200 × 400; initially 11.5 lb BW) were used in a 42-d study evaluating the effects of feeding Elarom SES in combination with high levels of ZnO and/or antimicrobials on nursery pig performance and fecal consistency. Elarom SES (Trouw Nutrition USA, Highland, IL) is a commercially available blend of short chain fatty acids (SCFAs), medium chain fatty acids (MCFAs) and slow release organic acids designed to enhance growth performance and gut health. Pigs were weaned at approximately 21 d and allotted to pens based on initial BW in a completely randomized design. Experimental treatments were arranged as a 2 × 2 × 2 factorial. The 8 treatment diets included: Elarom SES (none vs. 4 lb/ton), additional ZnO (none vs. 3,000 ppm in phase 1, 2,000 ppm in phase 2, and none in phase 3), and antimicrobial regimen (none vs. 400 g/ton CTC and 35 g/ton Denagard in Phase 1 and 50 g/ton Mecadox in Phases 2 and 3). Experimental diets were fed in 3 phases (Phase 1, d 0 to 7; Phase 2, d 7 to 21; and Phase 3, d 21 to 42 post-weaning) and fed in meal form. Overall, an Elarom SES × ZnO × antimicrobial interaction was observed for ADG ( $P = 0.043$ ) and F/G ( $P = 0.009$ ). The ADG interaction was the result of poorer ADG when Elarom SES or ZnO were added alone compared to when feed antimicrobials were added alone or when Elarom SES was added in combination with ZnO or ZnO was added in combination with antimicrobials. The F/G interaction was a result of the poorest F/G observed when all three additives were added in combination, compared to the control diet with Elarom SES or antimicrobials only and the diet with Elarom SES and ZnO in combination or the diet with ZnO and antimicrobial in combination. Adding antibiotics to the diet increased ( $P < 0.013$ ) ADG and ADFI, but there were no main effects of ZnO or Elarom SES observed. There were no individual or overall treatment effects ( $P > 0.100$ ), or treatment × day interactions ( $P = 0.53$ ) observed for fecal consistency. Overall, we observed some benefits in performance when adding combinations of ingredients compared to including them as stand alone products. More research should be conducted to confirm this response.

Key words: antimicrobial, Elarom SES, growth performance, nursery, zinc

<sup>1</sup> Appreciation is expressed to Dr. Kellie Hogan and Scott Webster, Trouw Nutrition USA, Highland, IL for their technical and partial financial support.

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## Introduction

With the introduction of new veterinary feed directive rules limiting the use of antimicrobials in swine feed, a need has arisen to find new alternatives that can replace the growth performance effects of feed grade antimicrobials. Elarom SES (Trouw Nutrition USA, Highland, IL) is a technology that combines the use of short chain fatty acids (SCFAs), medium chain fatty acids (MCFAs) and slow release organic acids that was developed and is sold by Trouw Nutrition. Initial research has shown that when Elarom SES is included in nursery pig diets, increased average daily gain and improved feed efficiency is observed compared to control diets without feed antibiotics. The effects of other feed additives on nursery growth performance such as dietary antibiotics and high levels of ZnO are well understood; however the effect of Elarom SES included in diets with or without these additives has not been characterized.

The objective of this study was to compare the growth performance and fecal consistency of nursery pigs fed diets containing antimicrobials, ZnO, and/or Elarom SES.

## Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 360 nursery pigs (DNA 200 × 400; initially 11.5 lb BW) were used in a 42-d study with 5 pigs per pen and 9 replications per treatment. Each pen had metal tri-bar flooring, one 4-hole self-feeder (4 ft. × 4 ft.) and a cup waterer to provide ad libitum access to feed and water. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW in a completely randomized design to 1 of 8 dietary treatments.

The 8 dietary treatments were based on a corn-soybean meal diet and arranged in a 2 × 2 × 2 factorial with main effects of added zinc from zinc oxide (ZnO; none vs. 3,000 ppm Zn from d 0 to 7, 2,000 ppm Zn from d 7 to 21, and no additional Zn above that provided in the trace mineral premix from d 21 to 42), feed antimicrobial (none vs. 400 g/ton CTC (Zoetis Services, LLC., Florham Park, NJ) and 35 g/ton Denagard (Elanco Animal Health, Greenfield, IN) from d 0 to 7 and 50 g/ton carbadox (Phibro Animal Health, Teaneck, NJ) from d 7 to 42), or Elarom SES (none vs. 4 lb/ton from d 0 to 42; Trouw Nutrition USA, LLC, Highland, IL). The treatment ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). Pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G.

Fecal scoring of pens occurred on d 0, 4, 7, 14, 21, 28, 35, and 42 by visual appraisal of the pen floor. Fecal scores were conducted before weighing on weigh days and were replicated by 3 individuals each day. Pens were scored on a scale from 1 to 5 with 1 indicating hard pellet type feces; 2 indicating firm, formed feces; 3 indicating soft, moist feces that retained shape; 4 indicating soft, unformed feces; and 5 indicating watery, liquid feces.

All diets were fed in meal form and were prepared at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Each diet contained 110 ppm of added Zn from ZnO from the trace mineral premix. Diet samples were collected at manufacturing, and pooled samples of each diet were submitted for analysis of DM, CP, Ca, P, and Zn (Ward Laboratories, Inc., Kearney, NE; Table 2). Analyzed diets confirmed diets manufactured with no added ZnO contained approximately 110 ppm ZnO from the trace mineral premix, and diets manufactured with added ZnO contained approximately 3,000 ppm ZnO in Phase 1 and approximately 2,000 ppm in Phase 2.

Growth data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The main effects of Zn, Elarom SES, and antimicrobial, as well as their interactions, were evaluated using preplanned CONTRAST statements. The main effects of Zn, Elarom SES, and antimicrobial, as well as day and treatment interactions were tested using preplanned CONTRAST statements. Fecal consistency scores were analyzed using PROC MIXED in SAS with pen as the experimental unit. The main effects of Zn, Elarom SES, and antibiotic, as well as day and treatment interactions were tested using preplanned CONTRAST statements. Differences between treatments were determined by using least squares means. A  $P$ -value  $\leq 0.05$  was considered significant and  $0.05 < P \leq 0.10$  was considered marginally significant.

## Results and Discussion

From d 0 to 7, an Elarom SES  $\times$  ZnO interaction ( $P = 0.016$ ) was observed for F/G (Table 3 and 4). The interaction occurred because pigs fed Elarom SES in combination with ZnO had improved F/G comparative to pigs fed diets containing only Elarom SES or ZnO with the control diet intermediate. Pigs fed diets containing feed antimicrobials had improved ADG ( $P = 0.047$ ) compared to those without.

From d 7 to 21, ADG tended to be greater ( $P = 0.091$ ) when diets contained Elarom SES, but ADFI and F/G were not influenced. Including ZnO in the diets increased ( $P < 0.001$ ) ADG, ADFI, and d 21 BW and marginally improved ( $P = 0.074$ ) F/G. Diets containing feed antimicrobials had improved ( $P < 0.001$ ) ADG, ADFI, F/G, and d 21 wt.

From d 0 to 21, an Elarom SES  $\times$  ZnO interaction was observed for F/G ( $P = 0.026$ ) and ADG ( $P = 0.053$ ). The greatest improvement in ADG and F/G was observed when both Elarom SES and ZnO were included in the diets compared to when either was added alone. Including ZnO in the diets increased ( $P < 0.001$ ) ADG, ADFI, and tended to improve ( $P = 0.071$ ) F/G. Diets containing feed antimicrobials had improved ( $P < 0.001$ ) ADG, ADFI, and F/G.

From d 21 to 42, an Elarom SES  $\times$  ZnO  $\times$  antimicrobial interaction tended to be observed ( $P = 0.055$ ) for ADG and was observed ( $P = 0.006$ ) for F/G. This interaction occurred because ADG and F/G was poorest when all three feed additives were added in combination compared to all other treatments. There was an Elarom SES  $\times$  antimicrobial interaction for ADFI ( $P = 0.013$ ) that was the result of ADFI being similar to control values when both additives were included; however, when only the antimicrobial was added to the diet, ADFI was increased.

Overall (from d 0 to 42), an Elarom SES  $\times$  ZnO  $\times$  antibiotic interaction was observed for ADG ( $P = 0.043$ ) and F/G ( $P = 0.009$ ). The ADG interaction was the result of poorer ADG when Elarom SES or ZnO were added alone compared to when antibiotic was added alone or when Elarom SES was added in combination with ZnO or ZnO was added in combination with antibiotic. The F/G interaction was a result of the poorest F/G being reported when all three additives were added in combination, compared to the control, diets with Elarom SES or antibiotic only and the diet with Elarom SES and ZnO in combination or the diet with ZnO and medication in combination. Overall, ADFI was increased ( $P < 0.001$ ) when antibiotic was included in the diets.

While there was no treatment effect on fecal consistency, there was a day effect observed ( $P = 0.001$ ; Table 5) resulting from d 0, 4, and 7 pigs exhibiting softer stool that improved to a firmer stool in the subsequent collection days.

In summary, these data suggest feeding combinations of different feed additives may influence growth performance in a manner that is different than their individual performance would indicate. For instance, the combination of Elarom SES and high ZnO had performance similar to the medication treatment, even though Elarom SES or high ZnO treatments fed alone had poorer performance than the medication treatment. The main effect of medication or ZnO during the Zn feeding phase was similar to previous studies. However, we unexpectedly observed poorer performance in some diets once ZnO was removed from the diet, which warrants further investigation. Also, further research should be considered to improve the understanding of feeding Elarom SES in combination with high ZnO and medication and at what periods of the nursery phase these additives should be fed to improve growth performance.

**Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	36.25	51.80	62.81
Soybean meal	20.65	27.25	32.57
Corn DDGS, 6-9% oil <sup>2</sup>	5.00	---	---
Blood plasma	4.00	---	---
Fish meal	1.25	1.25	---
Milk, whey powder	8.00	5.00	---
HP 300 <sup>3</sup>	5.00	5.00	---
Dairylac 80 <sup>4</sup>	15.00	---	---
CombiAcid <sup>5</sup>	0.20	0.20	---
Choice white grease	1.00	1.00	1.00
Limestone	1.03	1.00	1.08
Monocalcium phosphate, 21%	1.13	1.00	1.18
Sodium chloride	0.30	0.30	0.35
L-Lys HCl	0.30	0.38	0.35
DL-Met	0.17	0.20	0.14
L-Thr	0.10	0.15	0.13
L-Val	---	0.05	---
Elarom SES <sup>5,6</sup>	---	---	---
Zinc oxide <sup>6</sup>	---	---	---
Denagard <sup>6</sup>	---	---	---
CTC-50 <sup>6</sup>	---	---	---
Mecadox-2.5 <sup>6</sup>	---	---	---
Choline chloride, 60% liquid	0.04	---	---
Phytase <sup>7</sup>	---	0.02	0.02
Trace mineral premix <sup>8</sup>	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Total	100	100	100

*continued*

**Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Phase 1	Phase 2	Phase 3
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.40	1.35	1.25
Met:Lys	33	37	34
Met and Cys:Lys	57	58	57
Thr:Lys	62	63	62
Trp:Lys	19.3	17.8	18.1
Val:Lys	68	69	66
Total Lys, %	1.58	1.51	1.40
ME, kcal/lb	1,542	1,522	1,503
CP, %	22.8	22.2	21.2
Ca, %	0.80	0.74	0.7
P, %	0.75	0.67	0.65
Available P, %	0.51	0.47	0.42

<sup>1</sup>Phase 1 diet was fed from d 0 to 7 (~11.5 to 13.5 lb BW), Phase 2 diets from d 7 to 21 (~13.5 to 22.5 lb BW) and Phase 3 diets from d 21 to 42 (~22.5 to 46 lb BW).

<sup>2</sup>Dried distillers grains with solubles.

<sup>3</sup>Hamlet Protein, Inc., Findlay, OH.

<sup>4</sup>International Ingredients, Inc., St. Louis, MO.

<sup>5</sup>Trouw Nutrition USA, LLC., Highland, IL.

<sup>6</sup>Treatment diets contained zinc oxide added at 0 or 3,000 ppm from d 0 to 7 and at 0 or 2,000 ppm from d 7 to 21, Elarom SES (Trouw Nutrition USA, LLC., Highland, IL) added at either 0 or 0.2%, and medication regimen with 8 lb/ton CTC-50 (Zoetis Services, LLC., Florham Park, NJ) and 3.5 lb/ton Denagard (Elanco Animal Health, Greenfield, IN) added from d 0 to 7 and from d 7 to 42, 20 lb/ton Mecadox-2.5 (Phibro Animal Health, Teaneck, NJ). Additions of treatment ingredients were made in place of an equivalent amount of corn in respective experimental diets.

<sup>7</sup>HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), provided 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>8</sup>Trace mineral premix containing 17 ppm Cu and 110 ppm Zn.

**Table 2. Chemical analysis of experimental diets, %<sup>1</sup>**

Elarom SES:	-	+	-	-	+	+	-	+
Added ZnO:	-	-	+	-	+	-	+	+
Antimicrobial:	-	-	-	+	-	+	+	+
Phase 1 diets								
DM	91.0	91.2	91.4	91.2	91.0	91.1	91.0	91.4
CP	22.3	21.9	22.4	22.6	22.6	22.5	22.4	22.6
Ca	1.07	0.98	0.94	1.01	1.11	1.06	1.04	1.11
P	0.82	0.78	0.76	0.76	0.83	0.78	0.79	0.81
Zn, ppm	122	113	2,998	165	2,263	148	3,109	2,921
Phase 2 diets								
DM	89.0	88.4	89.7	88.9	89.5	89.3	90.0	88.8
CP	20.3	20.7	21.9	21.1	21.5	20.9	21.8	21.7
Ca	0.93	0.96	0.87	1.07	0.96	1.00	1.04	0.96
P	0.67	0.69	0.64	0.64	0.65	0.69	0.67	0.65
Zn, ppm	101	120	1,627	237	1,603	314	1,503	1,551
Phase 3 diets								
DM	88.1	88.1	88.1	87.6	88.1	88.2	87.6	88.2
CP	21.7	20.5	21.7	20.7	20.5	21.0	20.7	21.0
Ca	0.83	0.91	0.83	0.97	0.91	0.96	0.97	0.96
P	0.63	0.62	0.63	0.61	0.62	0.62	0.61	0.62
Zn, ppm	135	136	135	100	136	95	100	136

<sup>1</sup>Complete diet samples were obtained from each dietary treatment during manufacturing. Samples of diets were then submitted for analysis of DM, CP, Ca, P, and Zn (Ward Laboratories, Inc., Kearney, NE).

**Table 3. Effects of Elarom SES, ZnO, and/or antimicrobials on nursery pig performance <sup>1</sup>**

Elarom SES <sup>3</sup> :	-	+	-	-	+	+	-	+	
Added ZnO <sup>4</sup> :	-	-	+	-	+	-	+	+	
Antimicrobial <sup>5</sup> :	-	-	-	+	-	+	+	+	SEM
BW, lb									
d 0	11.5	11.5	11.5	11.5	11.5	11.5	11.6	11.6	0.081
d 7	13.4	13.2	13.4	13.7	13.5	13.3	13.7	13.5	0.143
d 21	21.1 <sup>ef</sup>	20.6 <sup>f</sup>	22.0 <sup>ed</sup>	23.0 <sup>bc</sup>	22.8 <sup>bcd</sup>	22.7 <sup>cd</sup>	23.7 <sup>ab</sup>	24.0 <sup>a</sup>	0.337
d 42	45.7 <sup>cd</sup>	44.9 <sup>d</sup>	44.4 <sup>d</sup>	48.4 <sup>ab</sup>	47.4 <sup>abc</sup>	46.2 <sup>abcd</sup>	48.6 <sup>a</sup>	46.0 <sup>bcd</sup>	0.882
d 0 to 7									
ADG, lb	0.26	0.24	0.27	0.31	0.28	0.26	0.30	0.28	0.02
ADFI, lb	0.29	0.29	0.33	0.31	0.29	0.30	0.32	0.31	0.015
F/G	1.10 <sup>ab</sup>	1.26 <sup>a</sup>	1.24 <sup>a</sup>	1.03 <sup>b</sup>	1.06 <sup>b</sup>	1.19 <sup>ab</sup>	1.05 <sup>a</sup>	1.12 <sup>ab</sup>	0.065
d 7 to 21									
ADG, lb	0.55	0.52	0.61	0.64	0.66	0.67	0.70	0.75	0.022
ADFI, lb	0.68	0.65	0.75	0.74	0.79	0.79	0.81	0.84	0.022
F/G	1.25	1.25	1.23	1.16	1.20	1.17	1.15	1.13	0.023
d 0 to 21									
ADG, lb	0.45 <sup>de</sup>	0.43 <sup>e</sup>	0.49 <sup>cd</sup>	0.53 <sup>c</sup>	0.53 <sup>bc</sup>	0.53 <sup>bc</sup>	0.57 <sup>ab</sup>	0.59 <sup>a</sup>	0.015
ADFI, lb	0.55	0.53	0.61	0.60	0.62	0.62	0.64	0.66	0.016
F/G	1.21 <sup>ab</sup>	1.24 <sup>a</sup>	1.23 <sup>a</sup>	1.13 <sup>c</sup>	1.17 <sup>bc</sup>	1.17 <sup>bc</sup>	1.13 <sup>c</sup>	1.12 <sup>c</sup>	0.02
d 21 to 42									
ADG, lb	1.17 <sup>a</sup>	1.16 <sup>a</sup>	1.05 <sup>b</sup>	1.21 <sup>a</sup>	1.18 <sup>a</sup>	1.12 <sup>ab</sup>	1.19 <sup>a</sup>	1.05 <sup>b</sup>	0.033
ADFI, lb	1.65 <sup>bc</sup>	1.64 <sup>bc</sup>	1.55 <sup>c</sup>	1.79 <sup>a</sup>	1.68 <sup>ab</sup>	1.68 <sup>ab</sup>	1.74 <sup>ab</sup>	1.66 <sup>b,c</sup>	0.042
F/G	1.41 <sup>d</sup>	1.42 <sup>d</sup>	1.48 <sup>bc</sup>	1.48 <sup>bc</sup>	1.44 <sup>cd</sup>	1.50 <sup>b</sup>	1.46 <sup>bcd</sup>	1.59 <sup>a</sup>	0.022
d 0 to 42									
ADG, lb	0.81 <sup>bc</sup>	0.79 <sup>c</sup>	0.77 <sup>c</sup>	0.86 <sup>ab</sup>	0.85 <sup>ab</sup>	0.82 <sup>abc</sup>	0.88 <sup>a</sup>	0.82 <sup>abc</sup>	0.02
ADFI, lb	1.10	1.09	1.08	1.18	1.15	1.14	1.18	1.16	0.026
F/G	1.36 <sup>c</sup>	1.37 <sup>bc</sup>	1.40 <sup>ab</sup>	1.37 <sup>bc</sup>	1.35 <sup>c</sup>	1.39 <sup>abc</sup>	1.35 <sup>c</sup>	1.42 <sup>a</sup>	0.015
Fecal consistency <sup>2</sup>	2.81	2.80	2.80	2.76	2.79	2.83	2.74	2.74	0.043

<sup>a,b,c</sup> Means within the same row with different superscripts differ ( $P \leq 0.05$ ).

<sup>1</sup>A total of 360 pigs (DNA 200 × 400) were used in a 3-phase nursery trial with 5 pigs per pen and 9 replications per treatment.

<sup>2</sup>Fecal consistency was categorized through scoring of consistency of feces from each pen (fecal scoring occurred on d 0, 4, 7, 14, 21, 28, 35, and 42). Pens were scored by 3 trained individuals; those 3 scores were then averaged and reported as pen means for overall and each collection day fecal consistency. Scoring scale guidelines: 1 = dry, firm pellet; 2 = firmly formed stool; 3 = soft stool that retains shape; 4 = soft, unformed stool; and 5 = watery liquid stool. There was no overall or individual treatment effect ( $P > 0.100$ ).

<sup>3</sup>Elarom SES (Trouw Nutrition USA, LLC, Highland, IL) added at 0.2% of the diet.

<sup>4</sup>Zinc oxide fed at 3,000 ppm in Phase 1 (d 0 to 7) and 2,000 ppm in Phase 2 (d 7 to 21).

<sup>5</sup>Phase 1: (400 g/ton CTC and 35 g/ton Denagard and 50 g/ton); Phases 2 and 3: (Mecadox 50 g/ton) (Phibro Animal Health, Teaneck, NJ).

**Table 4. Main and interactive effects of Elarom SES, added ZnO, and antimicrobials on nursery pig growth performance<sup>1,2</sup>**

	Probability, <i>P</i> <						
	Elarom SES	ZnO	Antimicrobial	Elarom SES × ZnO	Elarom SES × Antimicrobial	ZnO × Antimicrobial	Elarom SES × ZnO × Antimicrobial
<b>BW, lb</b>							
d 0	0.944	0.742	0.888	0.832	0.655	0.814	0.906
d 7	0.183	0.219	0.076	0.224	0.201	0.773	0.890
d 21	0.687	0.001	0.001	0.043	0.700	0.240	0.407
d 42	0.302	0.638	0.001	0.171	0.005	0.648	0.090
<b>d 0 to 7</b>							
ADG, lb	0.112	0.210	0.047	0.192	0.211	0.642	0.922
ADFI, lb	0.238	0.164	0.244	0.451	0.666	0.580	0.199
F/G	0.249	0.533	0.122	0.016	0.165	0.909	0.169
<b>d 7 to 21</b>							
ADG, lb	0.091	0.001	0.001	0.139	0.428	0.338	0.218
ADFI, lb	0.170	0.001	0.001	0.347	0.322	0.234	0.168
F/G	0.448	0.07	0.001	0.339	0.911	0.718	0.858
<b>d 0 to 21</b>							
ADG, lb	0.283	0.001	0.001	0.053	0.744	0.273	0.240
ADFI, lb	0.318	0.001	0.001	0.546	0.256	0.217	0.357
F/G	0.950	0.071	0.001	0.026	0.299	0.760	0.434
<b>d 21 to 42</b>							
ADG, lb	0.186	0.04	0.968	0.352	0.001	0.885	0.055
ADFI, lb	0.573	0.30	0.001	0.133	0.013	0.934	0.373
F/G	0.070	0.01	0.001	0.309	0.002	0.689	0.006
<b>d 0 to 42</b>							
ADG, lb	0.560	0.599	0.013	0.145	0.007	0.871	0.043
ADFI, lb	0.996	0.376	0.001	0.160	0.117	0.774	0.304
F/G	0.221	0.356	0.136	0.718	0.004	0.754	0.009

<sup>1</sup>A total of 360 pigs (DNA 200 x 400) were used in a 3-phase nursery trial with 5 pigs per pen and 9 replications per treatment.

<sup>2</sup>All experimental diets were fed in three phases (d 0 to 7, d 7 to 21, and d 21 to 42). All diets contained 110 ppm of Zn from the trace mineral premix.

**Table 5. Nursery pig fecal consistency over time**

Day	Fecal score <sup>1</sup>
0	3.4
4	3.4
7	3.3
14	2.3
21	2.1
28	2.4
35	2.6
42	2.8

<sup>1</sup>Fecal consistency scores were categorized by the consistency of feces per pen (fecal scores collected on d 0, 4, 7, 14, 21, 28, 35, and 42). Pens were scored by 3 trained individuals; those scores were then averaged and reported as pen means for each collection day. Scoring scale guidelines: 1 = dry, firm pellet; 2 = firmly formed stool; 3 = soft stool that retains shape; 4 = soft, unformed stool; and 5 = watery liquid. Treatment × Day interaction ( $P = 0.53$ ) and day effect ( $P < 0.01$ ).

## Effects of Increasing Copper from Tri-basic Copper Chloride or a Copper-Amino Acid Complex on Growth Performance of Nursery Pigs<sup>1</sup>

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### Summary

A total of 665 pigs [Group 1; 350 barrows (DNA 200 × 400; initially 14.1 lb)] and [Group 2; 315 barrows and gilts (DNA 241 × 600; initially 11.4 lb)] were used to determine the effects of added Cu source and level on nursery pig performance. There were 5 pigs per pen and 10 replications per treatment in group 1 and 5 pigs per pen and 9 replications per treatment in group 2. Pens of pigs were allotted by BW to 1 of 7 dietary treatments arranged as a 2 × 3 factorial plus a control diet, with main effects of Cu source (IntelliBond-C; Micronutrients, Indianapolis, IN or Mintrex-Cu; Novus, St. Charles, MO) and Cu level (75, 150, or 225 ppm). Diets were corn-soybean meal-based and were fed in meal form in 2 phases (d 0 to 14 and 14 to 35). All diets contained a trace mineral premix formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> in the complete diet.

Overall (d 0 to 35), there were no Cu source × level interactions observed. Increasing Cu increased ADG (linear,  $P = 0.048$ ) and final BW (linear,  $P = 0.019$ ). The increase in ADG with no effect on ADFI resulted in a tendency for improved F/G (linear,  $P = 0.091$ ) with increasing added Cu in the diet. There were no effects of Cu source on growth performance. Because the growth effects were linear, it is unknown from our study if increasing added Cu beyond 225 ppm would further improve growth.

Key words: nursery pig, copper, copper amino acid-complex, tri-basic copper chloride

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## Introduction

The NRC (2012<sup>4</sup>) reports weanling pigs have a nutritional Cu requirement of 6 ppm. However, according to Flohr et al. (2015<sup>5</sup>), many U.S. swine nutritionists formulate nursery pig diets to contain as low as 11 ppm and as high as 250 ppm Cu. Feeding high levels of added Cu (125 to 250 ppm) above that provided by the trace mineral premix has resulted in increased ADFI and ADG. Research has shown that despite dissimilar chemical structure of copper sources, nursery pig growth performance will be similar. Huang et al. (2015<sup>6</sup>) compared two inorganic sources [tri-basic copper chloride (TBCC) and CuSO<sub>4</sub>] of added Cu that ranged from 11 to 327 ppm in nursery pig diets and found growth benefits of feeding added Cu but no difference in growth between sources was observed.

Organic Cu sources are argued to be more bioavailable to the young pig due to their chemical structure, compared to inorganic sources. It has also been documented that both TBCC and Cu-AA Cu are more bioavailable than that of more typically used sources of Cu (Spears et al. 1997<sup>7</sup> and Wang et al. 2009<sup>8</sup>). Tri-basic copper chloride and Mintrex-Cu (Cu-AA) differ in their chemical characteristics. Tri-basic copper chloride is an inorganic mineral source, which is non-hygroscopic and poorly soluble in water but highly soluble in acidic conditions (Miles et al., 1998<sup>9</sup>). Mintrex-Cu is an organic form of Cu [Cu(HMTBa)<sub>2</sub>] which has been shown to be more bioavailable to the pig because of decreased binding activity with other dietary constituents, therefore suggesting less supplementation required compared with inorganic minerals in nursery pigs (Zhao et al., 2009<sup>10</sup>). Currently, to our knowledge, there are no data available that directly compare the effects of increasing added Cu from TBCC or Cu-AA on growth performance of nursery pigs. Therefore, our study was designed to investigate the effects of increasing Cu from either TBCC or Cu-AA added Cu source and level on growth performance of nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used for these studies. Two groups of pigs were used for the experiment. Group 1 pigs were housed at the K-State Segregated Early Weaning Facility in Manhattan, KS. Group 2 pigs were housed at the K-State Swine Teaching and Research Center

<sup>4</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

<sup>5</sup> Flohr, J. R.; Tokach, M. D.; Woodworth, J. C.; DeRouchey, J. M.; Dritz, S. S.; and Goodband, R. D. (2015) Vitamin and Trace Minerals: A Survey of Current Feeding Regimens, Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

<sup>6</sup> Y. L. Huang, M. S. Ashwell, R. S. Fry, K. E. Lloyd, W. L. Flowers, and J. W. Spears. 2015. Effect of dietary copper amount and source on copper metabolism and oxidative stress of weanling pigs in short-term feeding. *J. Anim. Sci.* 93:2948–2955.

<sup>7</sup> Spears, J. W., E. B. Kegley, L. A. Mullis, and T. A. Wise, 1997. Bioavailability of copper chloride in cattle. *J. Anim. Sci.* 75 (Suppl. 1):265. (Abstr.)

<sup>8</sup> Wang, Z., S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Evaluation of Mintrex copper as a source of copper in broiler diets. *Int. J. Poult. Sci.* 6:308-313.

<sup>9</sup> Miles, R. D., S. F. O'Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and prooxidant activity. *Poult. Sci.* 77:416–425. doi:10.1093/ps/77.3.416

<sup>10</sup> J. R. Zhao, J. Harrell, G. Allee, B. Hinson, P. Winkelbauer, C. Atwell, J. D. Richards, and M. Vazquez-Anon. 2009. Effect of an organic copper source on growth performance and tissue copper concentration in nursery pigs. Mid-west Animal Science Meeting, Des Moines, IA.

in Manhattan, KS. The research facilities were environmentally controlled. In group 1, each pen (3.9 × 4 ft) had tri-bar flooring and contained one 4-hole dry self-feeder and one cup waterer to provide ad libitum access to feed and water. For group 2, each pen (4 × 5 ft) had tri-bar flooring and contained one 4-hole dry self-feeder and one nipple waterer to provide ad libitum access to feed and water. Dietary treatments for each group were manufactured at the O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

In group 1, 350 barrows (DNA 200 × 400; initially 14.1 lb) were weaned at approximately 21 d of age and allotted to pen based on initial BW with 5 pigs per pen and 10 replicate pens per treatment. In group 2, 315 barrows and gilts (DNA 241 × 600; initially 11.4 lb) were weaned and allotted to pens based on initial BW and age. Age block 1 consisted of 4 replicate pens per treatment and pigs ranged in age from 16 to 20 d. Age block 2 consisted of 5 replicate pens per treatment and pigs ranged in age from 21 to 24 d. Group 1 and 2 pigs were fed a common starter diet for 7 and 5 d, respectively. On d 7 and 5 post-weaning for group 1 and 2, respectively, pens were allotted by BW to 1 of 7 dietary treatments arranged as a 2 × 3 factorial plus one control diet, with main effects of Cu source (IntelliBond-C; Micronutrients, Indianapolis, IN or Mintrex-Cu; Novus, St. Charles, MO) and Cu level (75, 150, or 225 ppm). Diets were corn-soybean meal-based and fed in meal form in 2 phases (d 0 to 14 and 14 to 35; Table 1). The trace mineral premix added to all diets provided complete diets with 17 ppm Cu from CuSO<sub>4</sub>.

In group 1 and 2, complete diet samples were collected from a minimum of 6 feeders and combined to make 1 composite sample per treatment and phase. Each sample was then split, ground, and sent to a commercial lab for analysis of DM, CP, Ca, P, ether extract, ash, and Cu concentrations. In group 1, all diets were analyzed at Cumberland Valley Analytical Services (Hagerstown, MD) and Ward Laboratories Inc. (Kearney, NE). Final Cu concentrations were determined by averaging 3 individual analyzed values, 2 from Cumberland Valley and 1 from Ward Labs. In group 2, all diets were analyzed at Cumberland Valley Analytical Services (Hagerstown, MD). Final Cu concentrations were determined by averaging 2 individual analyzed values.

For each group, pigs and feeders were weighed on d 0, 7, 14, 21, 28, and 35 to calculate ADG, ADFI, and F/G. Data were combined and analyzed as a randomized complete block design using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and dietary treatment as the fixed effect. Random effects of group and block within group were also used in the model. The main effects of source and level as well as their interaction were considered significant with  $P < 0.05$  and a tendency with  $P < 0.10$  and  $\geq 0.05$ ).

## Results and Discussion

The chemical analyses of the complete diets were similar to the intended formulation (Tables 2 and 3); however, the chemical analysis for Cu concentration was slightly higher than expected for diets formulated to contain 75 and 150 ppm of added Cu from Cu-AA (AAFCO, 2014<sup>11</sup>). Analytical variation for Cu is listed at 25% (AAFCO,

<sup>11</sup> Association of American Feed Control Officials (AAFCO). 2014. Official Publication. Assoc. Am. Feed Cont. Off., Champaign, IL.

2014). Total Ca and P concentrations were similar among diets across each dietary phase.

All other total Cu values for each diet in each group were within the acceptable analytical limits described by the Association of American Feed Control Officials (2014) given that 17 ppm of Cu from  $\text{CuSO}_4$  was provided by the trace mineral premix and accounting for the Cu provided by ingredients used in formulation. Corn (yellow dent) and soybean meal can contain on average 17 and 50 ppm Cu, respectively (NRC, 2012<sup>12</sup>). Based on these Cu concentrations, corn and soybean meal may have contributed up to 12 ppm to the complete diet in our study. Thus, some of the variation observed in the Cu analysis may partially be explained by the Cu concentrations provided by major ingredients used in formulation.

From d 0 to 14, there was a tendency for a source  $\times$  level interaction (quadratic,  $P = 0.086$ ; Table 4) for ADG with maximal ADG at 150 ppm Cu with the Cu-AA complex, but at 225 ppm with TBCC. For Cu main effects, increasing Cu increased (linear,  $P \leq 0.004$ ) ADG, ADFI, and d 14 BW with no changes in F/G.

From d 14 to 35, there were no Cu source  $\times$  level interactions observed. Neither Cu source nor level influenced ADG or ADFI; however, increasing Cu improved (linear,  $P = 0.035$ ) F/G.

Overall (d 0 to 35), there were no Cu source  $\times$  level interactions observed. Increasing Cu increased ADG (linear,  $P = 0.048$ ) and final BW (linear,  $P = 0.019$ ). The increase in ADG combined with no differences ADFI resulted in a tendency for improved F/G (linear,  $P = 0.091$ ).

It has been generally shown that increasing dietary Cu for nursery pigs increases ADG by increasing ADFI. Overall, increasing Cu increased ADG and tended to improve F/G but without any differences in ADFI. Interestingly, previous research has shown inconsistent performance differences in pigs fed different Cu sources. Unlike our study, previous research that compared TBCC or  $\text{CuSO}_4$  at increasing levels from 26 to 224 ppm found no response to the source or level of the Cu (De Jong et al., 2015<sup>13</sup>). The authors speculated their lack of response may have been related to the high levels of Zn that were fed in the diet immediately prior to their study; however, our study does not support this speculation. In each group of pigs for the study herein, pharmacological levels of Zn were fed prior to the pigs being fed experimental diets and a growth response was observed, particularly from d 0 to 14. Furthermore, because De Jong et al. (2015) did not observe any Cu response, they were unable to determine if one Cu source affected growth differently from the other.

In contrast, some earlier research reported improvements in ADG and F/G when pigs (initially 68.9 lb. BW), were fed either  $\text{CuSO}_4$  or TBCC compared with a control diet

<sup>12</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

<sup>13</sup> De Jong, J. A.; Bailey, L.; DeRouchey, J. M.; Tokach, M. D.; Goodband, R. D.; and Dritz, S. S. (2015). Effects of Copper Sources and Levels on Nursery Pig Growth Performance, Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

(Hastad et al., 2001<sup>14</sup>). In their study, the response to Cu was evident during the first 14 d, but not in the later part of the trial. The authors used increasing added Cu that ranged from 50 to 200 ppm and observed added Cu from either TBCC or CuSO<sub>4</sub> increased ADG compared with pigs fed the control diet; however, they observed growth was not linear as added Cu increased.

In our study, increasing Cu increased ADG and ADFI in the early nursery period but not the late nursery period. However, in each period the growth response magnitude to increasing level of Cu was numerically similar. In the early and late nursery period the ADG advantage was 0.05 and 0.04 lb/d, respectively, compared to the pigs fed the control diet. It appears that the ADG response to increasing levels of Cu was more difficult to detect in the late nursery period, which may be attributed to the increased amount of variation observed in that particular growth period. Additionally, because we observed a F/G response with no differences in feed intake during the late nursery period and increasing added Cu increased d 35 BW, this may help support that although not significant, there may be potential for a growth advantage to increasing added Cu during the late nursery period.

In summary, our study suggests that increasing TBCC or Cu-AA improves growth in nursery pigs. It appears the effect on ADG was more detectable during the early nursery period, with only a F/G improvement found during the second phase. The improved ADG led to a heavier BW both on d 14 and d 35. However, no differences between sources were observed. Because the growth effects were linear, it is unknown if increasing added Cu beyond 225 ppm would provide any further benefit for growth promotion above those observed in the current study.

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<sup>14</sup> Hastad, C. W. 2002. Phosphorus requirements of grow-finish pigs reared in commercial environments. MS Thesis. Kansas State University, Manhattan.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Phase	
	1	2
Ingredient, %		
Corn	48.47	57.30
Soybean meal (47.7% CP)	27.68	33.73
Dried whey	10.00	---
Choice white grease	5.00	5.00
Limestone	0.85	0.85
Monocalcium P (21% P)	1.60	1.70
Salt	0.30	0.35
L-Lys-HCl	0.33	0.33
L-Thr	0.15	0.16
HMB <sup>2</sup>	0.22	0.18
HP-300 <sup>3</sup>	5.00	---
Vitamin premix	0.15	0.15
Trace mineral premix	0.25	0.25
Cu source <sup>4,5</sup>	---	---
Total	100.00	100.00
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	1.30	1.25
Ile:Lys	63	62
Leu:Lys	122	124
Met: Lys	36	35
Met + Cys: Lys	58	58
Thr: Lys	65	65
Trp: Lys	18.4	18.4
Val: Lys	67	67
Total Lys, %	1.45	1.40
ME, kcal/lb	1,597	1,577
NE, kcal/lb	1,199	1,181
SID Lys:ME, g/Mcal	3.69	3.59
CP, %	21.7	21.3
Ca, %	0.85	0.80
P, %	0.78	0.75
Available P, %	0.49	0.44

<sup>1</sup>In each group of pigs, Phases 1 and 2 were fed from d 0 to 14 and 14 to 35, respectively. Dietary treatments were formed by adding 75, 150, or 225 ppm of Cu from either TBCC or Cu-AA at the expense of corn. The trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>Hydroxymethylthio-butanoic acid, Novus International (Saint Charles, MO).

<sup>3</sup>HP-300, Hamlet Protein, Findlay, OH, formulated with 3.25% SID lysine.

<sup>4</sup>Mintrex Cu, copper methionine hydroxy analogue (St. Charles, MO).

<sup>5</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).

**Table 2. Chemical analysis of diets, (Groups 1 and 2, as-fed basis)<sup>1</sup>**

Item	Phase 1							Phase 2						
	Added Cu, ppm							Added Cu, ppm						
	Control	TBCC, ppm <sup>2</sup>			Cu-AA, ppm <sup>3</sup>			Control	TBCC, ppm			Cu-AA, ppm		
	0	75	150	225	75	150	225	0	75	150	225	75	150	225
DM, % <sup>4</sup>	88.9	88.1	88.9	89.0	89.0	89.0	88.9	88.9	88.4	88.2	88.6	88.4	88.6	88.6
CP, % <sup>4</sup>	24.1	24.3	24.3	24.5	24.7	24.8	24.0	24.3	23.8	24.3	32.9	24.4	24.0	23.4
Crude fiber, % <sup>4</sup>	2.5	2.5	2.4	2.5	2.8	2.7	2.6	2.6	3.0	2.7	2.6	2.5	2.5	2.3
Ether extract, % <sup>4</sup>	6.0	6.7	6.4	7.2	6.9	6.8	7.3	7.3	7.1	7.0	7.0	7.3	7.1	6.6
Ash, % <sup>4</sup>	6.6	6.6	6.6	6.8	6.5	6.4	6.4	5.9	6.1	6.0	7.2	5.7	5.7	5.3
Ca, % <sup>4</sup>	1.06	0.97	1.01	1.01	0.94	0.95	1.03	1.02	0.93	0.93	1.01	0.93	0.99	0.93
P, % <sup>4</sup>	0.86	0.85	0.84	0.89	0.84	0.88	0.88	0.82	0.81	0.82	0.84	0.79	0.84	0.76
Cu, ppm <sup>4</sup>	24	86	179	248	134	227	316	28	90	144	246	114	177	283

<sup>1</sup>For each group of pigs, multiple samples of each diet were collected, blended and sub-sampled, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD). The trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).

<sup>3</sup>Mintrex-Cu, copper methionine hydroxy analogue (St. Charles, MO).

<sup>4</sup>Values represent the mean of analytical values for each group of pigs.

**Table 3. Effects of increasing Cu from TBCC or Cu-AA on growth performance of pigs<sup>1</sup>**

Item	Added Cu, ppm							SEM	Probability, <i>P</i> <				
	Control 0	TBCC <sup>2</sup>			Cu-AA <sup>3</sup>				Cu Source	Cu level		Source × level	
		75	150	225	75	150	225			Linear	Quadratic	Linear	Quadratic
BW, lb													
d 0	12.82	12.81	12.82	12.81	12.81	12.80	12.81	1.322	0.960	0.974	0.964	0.956	0.965
d 14	20.15	20.08	20.78	21.22	20.53	21.19	20.93	2.226	0.431	0.002	0.886	0.502	0.108
d 35	46.41	46.24	47.85	48.53	47.62	48.88	48.04	6.105	0.315	0.019	0.624	0.619	0.130
d 0 to 14													
ADG, lb	0.53	0.52	0.56	0.59	0.55	0.59	0.57	0.071	0.289	0.004	0.854	0.631	0.086
ADFI, lb	0.69	0.68	0.76	0.77	0.74	0.75	0.75	0.046	0.456	0.001	0.921	0.203	0.139
F/G	1.33	1.32	1.38	1.34	1.37	1.29	1.36	0.100	0.820	0.772	0.989	0.515	0.494
d 14 to 35													
ADG, lb	1.25	1.24	1.29	1.30	1.26	1.30	1.28	0.196	0.852	0.143	0.957	0.564	0.431
ADFI, lb	1.88	1.86	1.89	1.92	1.90	1.91	1.85	0.276	0.872	0.831	0.702	0.181	0.147
F/G	1.51	1.51	1.47	1.48	1.52	1.48	1.47	0.027	0.895	0.035	0.866	0.683	0.569
d 0 to 35													
ADG, lb	0.96	0.95	1.00	1.01	0.97	1.01	0.99	0.146	0.712	0.048	0.976	0.560	0.296
ADFI, lb	1.41	1.39	1.44	1.46	1.43	1.44	1.40	0.184	0.939	0.305	0.808	0.150	0.144
F/G	1.47	1.47	1.45	1.44	1.49	1.43	1.44	0.039	0.943	0.091	0.923	0.565	0.887

<sup>1</sup>A total of 665 pigs [Group 1; 350 barrows (DNA 200 × 400; initially 14.1 lb)] and [Group 2; 315 pigs (DNA 241 × 600; initially 11.4 lb)] were used in two 35-d growth studies. Data were combined across the 2 groups with 5 pigs per pen and 10 replications per treatment in group 1 and 5 pigs per pen and 9 replications per treatment in group 2. The treatment design was the same across both groups of pigs. In both groups of pigs the trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).

<sup>3</sup>Mintrex Cu, copper methionine hydroxy analogue (St. Charles, MO).

## Determination of Probiotic and/or Chlortetracycline Inclusion Effects on Nursery Pig Growth Performance<sup>1</sup>

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### Summary

A total of 300 nursery pigs (DNA 200 × 400, Columbus, NE; initially 13.0 lb BW) were used in a 42-d study evaluating the effects of feeding chlortetracycline (CTC) in combination with probiotics on nursery pig performance. Probiotics are a class of antimicrobial alternatives designed to enhance growth performance and digestive tract health. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW. Pigs were fed a common pelleted starter diet for 4 d and then weighed, and pens were allotted to 1 of 6 dietary treatments based on BW in a completely randomized design. The treatments were arranged in a 2 × 3 factorial with main effects of chlortetracycline (0 vs. CTC at 400 g/ton from d 0 to 42) and probiotic (0 vs. 1 lb/ton Bioplus 2B (Chr. Hansen USA, Inc., Milwaukee, WI)) vs. 1 lb/ton Poultry Star (Biomina America, Inc., San Antonio, TX). Experimental diets were fed in 2 phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 42) and fed in meal form. On d 14 and 28, CTC was removed from the diet according to FDA regulations. For overall performance, there were no interactions ( $P > 0.05$ ) between added probiotics and CTC. However, pigs fed CTC had improved ( $P < 0.001$ ) ADG, ADFI, and overall BW compared with those fed diets without CTC. While adding Poultry Star to the diet increased ( $P < 0.05$ ) BW and ADFI on d 14, there were no consistent benefits of feeding either probiotic alone or in combination with CTC.

Key words: antibiotic, growth performance, nursery, probiotic

### Introduction

The use of antimicrobials in feed and their positive benefits on growth performance during the nursery stage of weaned pig production is firmly established. In the past, producers widely used antimicrobials throughout the nursery stage of swine production, even in the absence of a health challenge. With the changing perspectives on the use of feed antimicrobials, alternative technologies are being considered that can possibly replace the growth performance benefits of feed grade antimicrobials.

<sup>1</sup> Appreciation is expressed to the National Pork Board for financial support.

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Probiotics are one such technology that enhances gut function for improved nutrient uptake. Therefore, the objective of this study was to compare the growth performance of nursery pigs fed diets containing antimicrobials and/or probiotics.

## Methods

This trial was conducted in collaboration with the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University. The main objective of the study was to evaluate the impact of probiotics on the emergence and dissemination of antimicrobial resistance among bacteria present in the gut. Two probiotics were chosen for our animal experiments based on our preliminary results on antimicrobial resistance carriage in them. Poultry Star (Biomim America, Inc., San Antonio, TX) was chosen in this study because the product contained resistance genes for 3 classes of antimicrobials. BioPlus 2B (Chr. Hansen USA, Inc., Milwaukee, WI) was chosen in this study because of the absence of antimicrobial resistance genes. This report describes the growth performance of these same pigs; the impact of these 2 probiotic sources on antimicrobial resistance will be reported elsewhere.

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 300 nursery pigs (DNA 200 × 400, Columbus, NE; initially 13.0 lb BW) were used in a 42-d study with 5 pigs per pen and 10 replications per treatment. Each pen (4 ft × 4 ft) had metal tri-bar flooring, one 4-hole self-feeder and a cup waterer to provide ad libitum access to feed and water. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW. Pigs were fed a common starter diet for 4 days and then allotted to 1 of 6 dietary treatments based on BW in a completely randomized design.

The 6 dietary treatments were based on a corn-soybean meal diet and arranged in a 2 × 3 factorial with main effects of antimicrobial (0 vs. chlortetracycline (CTC) at 400 g/ton from d 0 to 42; Zoetis Services, LLC., Florham Park, NJ), and probiotic (0 vs. 1 lb/ton BioPlus 2B; Chr. Hansen USA, Inc., Milwaukee, WI or 1 lb/ton Poultry Star; Biomim America, Inc., San Antonio, TX). The treatment ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). Experimental diets were fed in 2 phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 42) and fed in meal form. On d 14 and 28, CTC was removed from the diet according to FDA regulations. Experimental diets containing CTC resumed feeding on d 15 and 29. Pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G.

All experimental diets were fed in meal form and were prepared at the K-State O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. The 4 d common starter diet was fed in pellet form. Multiple diet samples were collected at manufacturing, and pooled samples of each diet were submitted for analysis of DM, CP, Ca, and P (Ward Laboratories, Inc., Kearney, NE; Table 2).

Growth data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The main effects of CTC and probiotics as well as their interactions, were evaluated using preplanned CONTRAST statements. These contrast statements were arranged as a  $2 \times 3$  factorial with the main effects of CTC and each of the probiotics. Differences between treatments were determined by using least squares means. A  $P$ -value  $\leq 0.05$  was considered significant and  $0.05 < P \leq 0.10$  was considered marginally significant.

## Results and Discussion

From d 0 to 14, a CTC  $\times$  Bioplus 2B interaction ( $P = 0.002$ ) was observed for ADFI (Table 4). The interaction occurred because pigs fed diets containing the combination of CTC and BioPlus 2B had greater ADFI compared to pigs fed the control diet or the diet with only BioPlus 2B, while pigs fed CTC intermediate. Pigs fed diets containing CTC had improved ( $P = 0.0001$ ) ADG and ADFI compared to those without.

From d 14 to 28, no interactions between CTC and either BioPlus 2B or Poultry Star were observed. Pigs fed diets with CTC had improved ( $P = 0.0001$ ) ADG, ADFI, and BW compared to pigs not fed CTC. Also, pigs fed diets containing Poultry Star had a tendency for greater ( $P = 0.052$ ) ADFI than those not fed Poultry Star.

From d 28 to 42, a CTC  $\times$  Poultry Star interaction ( $P = 0.050$ ) was observed for ADFI. The interaction occurred because pigs fed diets containing CTC only had greater ADFI compared to the control, while diets containing either Poultry Star or Poultry Star with CTC were intermediate. Furthermore, a tendency for a CTC  $\times$  BioPlus 2B interaction ( $P = 0.077$ ) was observed for F/G. The interaction occurred because pigs fed diets containing CTC in combination with BioPlus 2B had improved F/G comparative to pigs fed diets containing CTC or BioPlus 2B alone. Feeding CTC increased ( $P = 0.045$ ) ADFI, with no impact on ADG or F/G.

For the overall study (d 0 to 42), no CTC by probiotic interactions were observed. Pigs fed diets containing CTC had greater ( $P = 0.001$ ) ADG, ADFI, and overall BW compared to those not fed CTC.

In summary, feeding probiotics alone or in combination with CTC did not consistently improve nursery pig growth performance. This main effect of CTC on growth performance throughout the study was similar to previous research with an increase in growth rate driven by increased feed intake. In certain phases of the nursery, the addition of one of the probiotics (Poultry Star) with CTC had an additive effect on growth performance, but in later phases this benefit was not found. This warrants further research on whether in certain phases of nursery production it is beneficial to feed probiotics in combination with CTC to increase performance. In conclusion, this study further characterized the positive benefits of feeding CTC during the nursery phase on pig performance.

**Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>**

Item	Phase 1	Phase 2
Ingredient, %		
Corn	55.75	62.50
Soybean meal, 46.5% CP	25.35	33.40
Dried whey	10.00	---
HP 300 <sup>2</sup>	5.00	---
Limestone	1.05	1.18
Monocalcium phosphate, 21%	1.20	1.20
Sodium chloride	0.30	0.35
L-Lys HCl	0.45	0.45
DL-Met	0.20	0.20
L-Thr	0.20	0.20
L-Trp	0.03	0.03
L-Val	0.10	0.10
CTC-50 <sup>3</sup>	---	---
Bioplus 2B <sup>4</sup>	---	---
Poultry Star <sup>5</sup>	---	---
Phytase <sup>6</sup>	0.02	0.02
Trace mineral premix <sup>7</sup>	0.15	0.15
Vitamin premix	0.25	0.25
Total	100	100
Calculated analysis		
Standardized ileal digestible (SID) amino acids, %		
Lys	1.35	1.35
Met:Lys	36	36
Met and Cys:Lys	57	58
Thr:Lys	65	64
Trp:Lys	19.1	19.3
Val:Lys	70	70
Total Lys, %	1.49	1.50
ME, kcal/lb	1,496	1,482
CP, %	21.4	21.9
Ca, %	0.75	0.75
P, %	0.69	0.66
Available P, %	0.49	0.43

<sup>1</sup>Phase 1 diets were fed from d 0 to 14 (~13.0 to ~19 lb BW) and Phase 2 diets from d 14 to 42 (~19 to 55 lb BW). A common starter diet was fed to all pigs for 4 days after weaning.

<sup>2</sup>Hamlet Protein, Inc., Findlay, OH.

<sup>3</sup>Zoetis Services, LLC., Florham Park, NJ.

<sup>4</sup>Chr. Hansen USA, Inc., Milwaukee, WI.

<sup>5</sup>Biomim America, Inc., San Antonio, TX.

<sup>6</sup>HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>7</sup>Trace mineral premix containing 17 ppm Cu and 110 ppm Zn.

**Table 2. Diet analysis, %<sup>1</sup>**

CTC	-	+	-	+	-	+
Bioplus 2B	-	-	+	+	-	-
Poultry Star	-	-	-	-	+	+
Phase 1 diets						
DM	89.5	89.5	90.1	89.9	89.7	89.2
CP	21.1	21.4	21.3	21.8	21.8	21.1
Ca	0.85	0.91	0.93	1.05	0.86	0.94
P	0.74	0.70	0.72	0.70	0.73	0.69
Phase 2 diets						
DM	88.0	88.3	88.0	88.2	88.6	88.9
CP	21.7	20.7	21.5	20.8	21.0	21.8
Ca	0.85	0.99	0.96	1.05	0.95	1.08
P	0.66	0.69	0.67	0.68	0.69	0.70

<sup>1</sup>Complete diet samples were obtained from each treatment during manufacturing and composited. Samples of diets were then submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis.



## Evaluating the Effects of Replacing Feed Grade Antibiotics with Yeast, Cinnamon, or Zinc Oxide and Copper Sulfate on Nursery Pig Performance

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### Summary

A total of 288 weaned pigs (Line 200 × 400; DNA, Columbus, NE; initially 11.8 lb) were used in a 42-d study to compare the effects of feeding antibiotic alternatives (pharmacological trace minerals, copper and zinc, yeast, or essential oils), alone or in combination, on nursery pig performance in replacement to a common antimicrobial agent (carbadox, Mecadox<sup>®</sup>, Phibro Animal Health, Teaneck, NJ). Pigs were allotted to 1 of 9 dietary treatments in pens of 4 at weaning in a randomized complete block design with 8 replications per treatment. Dietary treatments were arranged with a negative control diet with no medication or other feed additive, a positive control with added carbadox, or 7 treatments including added copper sulfate ( $\text{CuSO}_4$ ; 0 vs. 125 ppm Cu) and added zinc oxide ( $\text{ZnO}$ ; 0 vs. 3,000 ppm Zn from d 0 to 7 and 2,000 ppm Zn from d 7 to 28), essential oils from XTRACT 6930 (*Capsicum oleoresin* 2%, carvacrol 5%, cinnamaldehyde 3%, Pancosma North America, Drummondville, Quebec, Canada) at 2 lb/ton, Safmannan A (Yeast cell walls, Lesaffre Yeast Corporation, Milwaukee, WI) at 0.5 lb/ton, and Actisaf HR (yeast cells, Lesaffre Yeast Corporation, Milwaukee, WI) at 1.5 lb/ton. These supplements were fed alone or in combination. From d 0 to 7 experimental diets were a pelleted ration; and fed in a meal form from d 7 to 28, followed by a common corn-soybean meal-based diet without any antimicrobial, pharmacological trace minerals, essential oils, or yeast from d 28 to 42. Essential oils and yeast had no significant ( $P > 0.05$ ) effect on ADG. Feeding carbadox or pharmacological trace minerals (Cu and Zn) improved ADG ( $P < 0.05$ ) of nursery pigs compared to the non-medicated control diet. Carryover effects from any of these dietary treatments on subsequent growth performance were not significantly different ( $P > 0.05$ ). The use of pharmacological trace minerals Cu and Zn alone or in conjunction with either yeast or essential oil allows for competitive ADG and F/G with an antimicrobial agent, like carbadox. In summary, under the conditions of this experiment, pigs fed the combination of zinc and copper had similar growth performance to those fed carbadox ( $P > 0.05$ ).

Key words: alternative, antibiotic, carbadox, copper, essential oil, nursery pig, pharmacological trace minerals, yeast, zinc

## Introduction

Since feed-grade antibiotics became available to the swine industry in the mid-1950s, research has shown that dietary inclusions of these antimicrobial agents improve growth rate and feed efficiency of nursery pigs. Recently, considerable discussion has been focused on the potential of antimicrobial resistance and its potential ties to feeding antimicrobial agents to swine. The push to eliminate use of antimicrobial agents for growth promotion is a primary objective of the swine industry. Many swine producers have shared their concern with possible production losses caused by the elimination of antimicrobial agents' use in swine diets, particularly in the nursery phase. Thus, we conducted this experiment because of three critical points. First, as consumers rightfully become more concerned, major retailers and meat producing companies are taking a proactive stance on antibiotic-free pork. Secondly, several classes of feed additives have been postulated to be able to replace antimicrobial agents in nursery diets. These include, but are not exclusive to the following classes of compounds; phytochemicals (essential oils), yeast and yeast cell walls, pharmacological levels of trace minerals, and combinations of these additives. Lastly, because of initial research by Feldpausch we hypothesized that feeding a combination of these feed additives would provide a direct comparison to a popular feed antimicrobial, carbadox, on nursery pig performance. Therefore, the objective of this experiment was to compare the growth performance of nursery pigs fed diets containing carbadox and different dietary supplements that are commonly fed as antibiotic alternatives (pharmacological levels of Zn and Cu, essential oils, and yeast), alone or in combination with each other.

## Procedures

This trial was conducted as a follow-up study to Feldpausch with the primary objective of evaluating the potential impact of different types of feed additives used as antibiotic alternatives in replacing antimicrobial agents. This report describes the growth performance of nursery pigs and the effects of antibiotic alternatives on growth performance.

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the K-State Swine Teaching and Research farm nursery in Manhattan, KS.

A total of 288 nursery pigs (Line 200 × 400; DNA, Columbus, NE; initially 11.8 lb BW) were used in a 42-d study with 4 pigs per pen and 8 replications per treatment. Each pen had one 4-hole self-feeder, metal tri-bar flooring, and a nipple waterer to provide ad libitum access to feed and water. Pigs were weaned at approximately 21 d of age. Allotment divided the pigs into heavy and light blocks based on initial BW. Pigs were grouped to achieve equal average pen weights. Based on d 0 weights, pigs were randomly allotted to 1 of 9 dietary treatments in blocks by barn location. The 9 dietary treatments consisted of a corn-soybean meal-based diet and were arranged with treatments of pharmacological trace minerals with added Cu from copper sulfate ( $\text{CuSO}_4$ ; 0 vs. 125 ppm Cu) and added Zn from zinc oxide ( $\text{ZnO}$ ; 0 vs. 3,000 ppm Zn from d 0 to 7 and 2,000 ppm Zn from d 7 to 28), essential oil blend from XTRACT 6930 (*Capsicum oleoresin* 2%, carvacrol 5%, cinnamaldehyde 3%, Pancosma North America, Drummondville, Quebec, Canada) from d 0 to 28 at 2 lb/ton, Safmannan A (Yeast cell walls, Lesaffre Yeast Corporation, Milwaukee, WI) from d 0 to 28 at 0.5 lb/ton, Actisaf

HR (yeast cells, Lesaffre Yeast Corporation, Milwaukee, WI) from d 0 to 28 at 1.5 lb/ton, and carbadox (Mecadox®, Phibro Animal Health, Teaneck, NJ) from d 0 to 28 at 50 g/ton. Equivalent amounts of corn were replaced with treatment diet to form the experimental treatments.

The experimental diets were fed from d 0 to 28. Phase 1 experimental diets were a pelleted ration fed from d 0 to 7 (Table 1). All diets had an acidifier (Kem-Gest, Kemin, Des Moines, IA) at 0.4 lb/ton added to diets during the Phase 1 period. Phase 2 experimental diets were fed in meal form, from d 7 to 28 (Table 2). From d 28 to 42, pigs were fed a common Phase 3 meal feed diet, (Table 3) until the completion of the trial on d 42. No medication, yeast, essential oils, or pharmacological levels of Cu or Zn were fed to all pigs, during this period, to evaluate any carryover effects from the treatment diets.

All diets were prepared at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Diet samples were collected periodically throughout the study and pooled samples of each diet were analyzed. Average daily gain, ADFI, and F/G were determined by weighing pigs and measuring feed disappearance on d 7, 14, 21, 28, 35, and 42.

Growth data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS with pen as the experimental unit. The model included the main effect of Cu from CuSO<sub>4</sub>, Zn from ZnO, essential oils from XTRACT 6930, yeast from Safmannan A and Actisaf HR and carbadox, and weight block as a random effect. The Kenward-Roger adjustment was used for denominator degrees of freedom. Differences between treatments were determined by using the p-value difference option. Least squares means were considered significantly different at  $P \leq 0.05$  and a trend at  $P \leq 0.10$ .

## Results and Discussion

During Phases 1 and 2 of the experiment (d 0 to 7 and 7 to 28), pigs fed carbadox proved to have increased ADG ( $P < 0.05$ ) compared to pigs fed a non-medicated control diet. This resulted in greater d-28 BW ( $P < 0.05$ ). When carbadox was removed and pigs were fed a common diet from d 28 to 42 there was no significant difference in ADG ( $P > 0.05$ ) compared to pigs fed a non-medicated control diet, ending in no effect overall from d 0 to 42 by carbadox. Carbadox did not improve feed efficiency during the entire experimental trial ( $P=0.08$ ).

During the experimental treatment period, yeast alone or essential oils alone did not improve ( $P > 0.05$ ) growth performance compared to those fed a non-medicated control diet. However, pigs fed pharmacological trace minerals (Cu and Zn) alone had similar growth performance to those fed carbadox during d 0 to 28 ( $P > 0.05$ ). Additionally, pigs fed pharmacological levels of trace minerals in combination with yeast, essential oils or in combination with both yeast and essential oils also showed comparable ( $P > 0.05$ ) growth performance to those pigs fed carbadox during Phases 1 and 2. Throughout the common diet phase from d 28 to 42, there were no differences in ADG, ADFI, and F/G for any dietary treatments. Overall, d 0 to 42, ADG, ADFI, and F/G of pigs fed pharmacological trace minerals alone had equal ( $P > 0.05$ ) growth per-

formance with those fed carbadox. However, pigs fed pharmacological levels of Zn and Cu outperformed control pigs during this period. These trace minerals alone increased d-42 weights (49.4 lb.), compared to carbadox-fed pigs with d-42 weights (47.1 lb). The overall positive effects of combining Zn and Cu resulted in an average of a 5 lb per pig increase in weight at d-42 post-weaning compared to pigs fed control diets.

Typically, nursery pigs are fed a diet containing an antimicrobial agent. We fed carbadox to nursery pigs and found a consistent improvement in growth performance compared to pigs fed a non-medicated diet. However, feeding antibiotics to pigs is under increased scrutiny. Thus, our industry must research dietary ingredients that could be used as antibiotic alternatives.

Pharmacological levels of Cu and Zn are typically added during different dietary phases of the nursery period. Typically, Zn is added to diets fed to nursery pigs during early nursery period (d 0 to 14) and Cu during the later period (d to 14 to 42). Zinc oxide (ZnO) is the most common form of added dietary Zn, while Cu most commonly comes from copper sulfate. In our current experiment, we added zinc oxide and copper sulfate in combination to diets for nursery pigs. Pigs fed the Zn and Cu combination had equal to or even greater performance to pigs fed carbadox. In addition, pigs fed the zinc oxide and copper sulfate combination were over 5 pounds heavier ( $P < 0.0081$ ) at the end of the nursery phase (d-42) compared to pigs fed a non-medicated control diet.

Finally, we investigated the effects of two popular ingredients that have been postulated to enhance nursery pig growth performance, as possible antibiotic replacements. When feeding yeast or essential oils alone or in combination, we found no consistent effects on nursery pig growth performance. Finally, we fed yeast or essential oils in combination with the mineral-supplemented treatment ( $\text{CuSO}_4$  and ZnO). No further benefit in growth performance was seen, beyond the benefits of adding supplemental Cu and Zn, was found by adding yeast or essential oil. The benefits in growth performance was nearly entirely due to Zn and Cu supplementation during the feeding trial on nursery pigs.

In summary, we are optimistic that under the conditions of this experiment that a mineral combination of zinc oxide and copper sulfate could be an effective replacement for carbadox in diets fed to nursery pigs.

**Table 1. Composition of Phase 1 diets<sup>1</sup>**

Ingredient, %	A <sup>2</sup>	B <sup>3</sup>	C <sup>4</sup>	D <sup>5</sup>	E <sup>6</sup>	F <sup>7</sup>	G <sup>8</sup>	H <sup>9</sup>	I <sup>10</sup>
Corn	37.35	36.35	37.25	36.90	37.30	36.80	36.85	37.20	36.75
Soybean meal	19.85	19.85	19.85	19.85	19.85	19.85	19.85	19.85	19.85
Blood meal	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Blood plasma	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Corn DDGs, > 6 & < 9% Oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Milk, whey powder	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Choice white grease	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Limestone, ground	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys-HCL	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
DL-Met	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Thr	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride 60%	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Kemgest	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mecadox	---	1.00	---	---	---	---	---	---	---
Biosaf-HR	---	---	0.08	---	---	0.08	---	0.08	0.08
Safman	---	---	0.03	---	---	0.03	---	0.03	0.03
Copper sulfate (125 ppm)	---	---	---	0.05	---	0.05	0.05	---	0.05
Zinc oxide	---	---	---	0.42	---	0.42	0.42	---	0.42
XTRACT 6930	---	---	---	---	0.05	---	0.05	0.05	0.05

<sup>1</sup>All Phase I diets were pelleted.

<sup>2</sup>No antibiotic-negative control (with organic acid Phase 1 only, Phase 2, and Phase 3, no acid).

<sup>3</sup>Positive control-Mecadox 50 g/ton.

<sup>4</sup>N.C. + yeast (1.5 lb/ton Biosaf-HR; 0.5 lb/ton Safman).

<sup>5</sup>N.C. + Zinc oxide (Phase 1; 3,000 ppm d 0 to 7; Phase 2; 2,000 ppm d 7 to 28) and copper sulfate (125 ppm).

<sup>6</sup>N.C. + XTRACT 6930 (2.0 lb/ton).

<sup>7</sup>N.C. + ZnO and CuSO<sub>4</sub> + yeast.

<sup>8</sup>N.C. + ZnO and CuSO<sub>4</sub> + XTRACT 6930.

<sup>9</sup>N.C. + yeast + XTRACT 6930.

<sup>10</sup>N.C. + ZnO and CuSO<sub>4</sub> + yeast + XTRACT 6930.

**Table 2. Composition of Phase 2 diets<sup>1</sup>**

Ingredient, %	A <sup>2</sup>	B <sup>3</sup>	C <sup>4</sup>	D <sup>5</sup>	E <sup>6</sup>	F <sup>7</sup>	G <sup>8</sup>	H <sup>9</sup>	I <sup>10</sup>
Corn	54.71	53.71	54.61	54.38	54.66	54.28	54.33	54.56	54.23
Soybean meal	29.55	29.55	29.55	29.55	29.55	29.55	29.55	29.55	29.55
Blood meal	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Fish meal	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Milk, whey powder	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Monocalcium	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Limestone, ground	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys-HCL	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Met	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Thr	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HiPhos 2700	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mecadox	---	1.00	---	---	---	---	---	---	---
Biosaf-HR	---	---	0.08	---	---	0.08	---	0.08	0.08
Safman	---	---	0.03	---	---	0.03	---	0.03	0.03
Copper sulfate (125 ppm)	---	---	---	0.05	---	0.05	0.05	---	0.05
Zinc oxide	---	---	---	0.28	---	0.28	0.28	---	0.28
XTRACT 6930	---	---	---	---	0.05	---	0.05	0.05	0.05

<sup>1</sup>All Phase II rations were meal diets.

<sup>2</sup>No antibiotic-negative control (with organic acid Phase 1 only, Phase 2 and Phase 3, no acid).

<sup>3</sup>Positive control-Mecadox 50 g/ton.

<sup>4</sup>N.C. + yeast (1.5 lb/ton Biosaf-HR; 0.5 lb/ton Safman).

<sup>5</sup>N.C. + Zinc oxide (Phase 1; 3,000 ppm d 0 to 7; Phase 2; 2,000 ppm d 7 to 28) and copper sulfate (125 ppm).

<sup>6</sup>N.C. + XTRACT 6930 (2.0 lb/ton).

<sup>7</sup>N.C. + ZnO and CuSO<sub>4</sub> + yeast.

<sup>8</sup>N.C. + ZnO and CuSO<sub>4</sub> + XTRACT 6930.

<sup>9</sup>N.C. + Yeast + XTRACT 6930.

<sup>10</sup>N.C. + ZnO and CuSO<sub>4</sub> + yeast + XTRACT 6930.

**Table 3. Composition of Phase 3 Diets**

Ingredient, %	Phase 3 <sup>1</sup>
Corn	63.83
Soybean meal	32.85
Monocalcium	1.00
Limestone, ground	1.03
Sodium chloride	0.35
L-Lys-HCL	0.30
DL-Met	0.12
L-Thr	0.12
Trace mineral premix	0.15
Vitamin premix	0.25
HiPhos 2700	0.02

<sup>1</sup>All treatments were fed a common corn-soybean meal, meal feed from d 28 to 42.

**Table 4. Effects of pharmacological trace minerals, essential oils, yeast, and carbadox on nursery pig growth performance<sup>1,2</sup>**

Yeast/yeast cell walls <sup>3</sup> :	-	-	+	-	-	+	-	+	+	
Added Cu/Zn <sup>4</sup> :	-	-	-	+	-	+	+	-	+	
Essential oil blend <sup>5</sup> :	-	-	-	-	+	-	+	+	+	
Carbadox <sup>6</sup> :	-	+	-	-	-	-	-	-	-	SEM
<b>BW, lb</b>										
d 0	11.8	11.8	11.8	11.9	11.8	11.8	11.8	11.8	11.8	0.6006
d 7	12.8	13.6	12.9	13.8	13.0	14.0	13.7	13.2	13.5	0.6815
d 14	14.8	16.7	14.6	16.9	15.2	16.9	17.0	15.6	16.3	0.8406
d 28	27.6	30.5	27.6	32.5	28.1	30.8	31.0	28.8	30.3	1.3048
d 42	44.3	47.1	46.0	49.4	46.6	48.9	48.8	45.8	47.9	2.0132
<b>d 0 to 7</b>										
ADG, lb	0.15	0.26	0.15	0.27	0.16	0.33	0.27	0.21	0.25	0.0286
ADFI, lb	0.60	0.60	0.63	0.55	0.64	0.55	0.60	0.56	0.57	0.2384
F/G	3.99	2.30	4.23	2.03	3.98	1.67	2.24	2.69	2.28	0.3543
<b>d 7 to 14</b>										
ADG, lb	0.28	0.45	0.25	0.43	0.33	0.41	0.47	0.33	0.40	0.03672
ADFI, lb	0.60	0.64	0.52	0.66	0.53	0.67	0.58	0.57	0.62	0.03725
F/G	2.14	1.43	2.07	1.54	1.62	1.63	1.22	1.71	1.56	0.5007
<b>d 0 to 14</b>										
ADG, lb	0.22	0.35	0.20	0.35	0.24	0.37	0.37	0.27	0.32	0.02736
ADFI, lb	0.60	0.62	0.58	0.60	0.58	0.61	0.59	0.57	0.59	0.02154
F/G	2.73	1.76	2.89	1.73	2.42	1.64	1.60	2.10	1.86	0.2633
<b>d 7 to 28</b>										
ADG, lb	0.70	0.81	0.70	0.84	0.72	0.80	0.82	0.74	0.80	0.03606
ADFI, lb	1.26	1.40	1.19	1.43	1.19	1.41	1.34	1.26	1.38	0.05133
F/G	1.79	1.73	1.70	1.70	1.65	1.78	1.63	1.69	1.72	0.06045
<b>d 28 to 42</b>										
ADG, lb	1.19	1.18	1.32	1.28	1.32	1.29	1.27	1.21	1.26	0.07348
ADFI, lb	1.91	1.95	1.97	1.92	1.89	1.92	1.94	1.94	1.92	0.05828
F/G	1.61	1.65	1.50	1.49	1.43	1.49	1.53	1.60	1.53	0.09207
<b>d 0 to 42</b>										
ADG, lb	0.77	0.84	0.81	0.89	0.83	0.88	0.88	0.81	0.86	0.03884
ADFI, lb	1.37	1.45	1.36	1.45	1.33	1.44	1.42	1.37	1.42	0.03988
F/G	1.77	1.73	1.67	1.62	1.60	1.63	1.61	1.69	1.65	0.05839

<sup>1</sup>A total of 288 nursery pigs (DNA Line 200 × 400, initially 11.8 lb BW) were used in a 42-day study with 4 pigs per pen and 8 replications per treatment.

<sup>2</sup>Experimental treatment diets were fed from d 0 to d 28. All treatments were fed a common diet from d 28 to d 42.

<sup>3</sup>Yeast and yeast cell walls were added as (1.5 lb/ton of Biosaf-HR; 0.5 lb/ton Safmannan).

<sup>4</sup>Pharmacological trace minerals; Cu from CuSO<sub>4</sub> was added to treatment diets at 0 vs. 125 ppm, and Zn from ZnO at 3,000 ppm from d 0 to 7 and 2,000 ppm from d 7 to 28.

<sup>5</sup>Essential oils were added as XTRACT 6930 at 2 lb/ton (*Capsicum oleoresin* 2%, carvacrol 5%, cinnamaldehyde 3%, hydrogenated rapeseed oil 90%) from d 0 to 28.

<sup>6</sup>Mecadox<sup>®</sup> was added at either 0 or 50 g/ton from d 0 to 28.

<sup>7</sup>Caloric efficiency is expressed as kcal per pound of live weight gain.

**Table 5. Statistical analysis of dietary alternatives, pharmacological trace minerals Cu and Zn, essential oils, yeast, and carbadox on nursery pig growth performance<sup>1</sup>**

	Probability, P <												
	Control × Mecadox	Control × Cu/Zn	Mecadox × Yeast	Mecadox × Cu/Zn	Mecadox × Essential Oils (EO)	Mecadox × Cu/Zn + Yeast	Mecadox × Cu/Zn + EO	Mecadox × Yeast + EO	Mecadox × Yeast + EO	Mecadox × Cu/Zn × Yeast	Cu/Zn × Cu/Zn + Yeast	Cu/Zn × Cu/Zn + EO	Cu/Zn × Cu/Zn + Yeast + EO
BW, lb													
d 0	0.8468	0.0971	0.9230	0.2340	0.7280	0.8468	0.6993	0.9230	0.8468	0.1450	0.1572	0.1450	
d 7	0.0073	0.0004	0.0100	0.3145	0.0218	0.0821	0.6387	0.1916	0.8325	0.4532	0.5893	0.2248	
d 14	0.0002	< 0.0001	< 0.0001	0.7643	0.0030	0.6862	0.5151	0.0192	0.3838	0.9169	0.7249	0.2434	
d 28	0.0158	0.0001	0.0138	0.0947	0.0407	0.8279	0.6903	0.1524	0.8636	0.1440	0.1991	0.0663	
d 42	0.1345	0.0081	0.5569	0.2246	0.7986	0.3312	0.3490	0.5078	0.6501	0.8054	0.7780	0.4435	
d 0 to 7 <sup>2</sup>													
ADG, lb	0.0083	0.0023	0.0098	0.6538	0.0206	0.0767	0.6771	0.1921	0.8980	0.1816	0.9744	0.5645	
ADFI, lb	0.5443	0.1602	0.0726	0.4195	0.2432	0.1488	0.7157	0.7461	0.7461	0.5179	0.2432	0.6274	
F/G	0.0018	0.0024	0.1524	0.9207	0.3497	0.8246	0.8246	0.4820	0.7219	0.7482	0.7482	0.7976	
d 7 to 14													
ADG, lb	0.0008	0.0028	< 0.0001	0.6874	0.0099	0.3918	0.5734	0.0161	0.2736	0.6484	0.3358	0.4861	
ADFI, lb	0.5710	0.9247	0.1399	0.6367	0.1887	0.7587	0.3343	0.0689	0.3962	0.8686	0.6200	0.7055	
F/G	0.0367	0.0117	0.0005	0.6437	0.1536	0.7791	0.3394	0.3907	0.9047	0.8552	0.6200	0.7315	

*continued*

**Table 5. Statistical analysis of dietary alternatives, pharmacological trace minerals Cu and Zn, essential oils, yeast, and carbadox on nursery pig growth performance<sup>1</sup>**

	Probability, P <											
	Control × Mecadox	Control × Cu/Zn	Mecadox × Yeast	Mecadox × Cu/Zn	Mecadox × Essential Oils (EO)	Mecadox × Cu/Zn + Yeast	Mecadox × Cu/Zn + EO	Mecadox × Yeast + EO	Mecadox × Yeast + EO	Mecadox × Cu/Zn × Cu/Zn × Yeast	Mecadox × Cu/Zn × Cu/Zn × EO	Mecadox × Cu/Zn × Cu/Zn × Yeast + EO
d 0 to 14												
ADG, lb	0.0002	0.0003	< 0.0001	0.9711	0.0028	0.6905	0.6125	0.0164	0.3863	0.6641	0.5874	0.4062
ADFI, lb	0.3828	0.3828	0.0188	1.0000	0.0580	0.6304	0.3161	0.1190	0.5406	0.6304	0.3161	0.5406
F/G	< 0.0001	< 0.0001	0.0003	0.8556	0.0215	0.5830	0.3294	0.1838	0.7654	0.7132	0.4265	0.6311
d 7 to 28 <sup>3</sup>												
ADG, lb	0.0266	0.0036	0.0189	0.4510	0.0574	0.8012	0.6954	0.1494	0.8667	0.3159	0.7161	0.3575
ADFI, lb	0.0299	0.0019	0.0036	0.3061	0.0034	0.8560	0.6006	0.0476	0.6870	0.3986	0.1245	0.1560
F/G	0.3802	0.4379	0.8258	0.9182	0.4913	0.7030	0.3647	0.8258	0.8258	0.7805	0.3135	0.7469
d 28 to 42												
ADG, lb	0.9303	0.2641	0.1192	0.2291	0.1034	0.1976	0.2960	0.7047	0.3753	0.9303	0.8726	0.7484
ADFI, lb	0.2860	0.7126	0.0304	0.4826	0.0054	0.6878	1.0000	0.4621	0.6393	0.2714	0.4826	0.2437
F/G	0.4137	0.1173	0.5156	0.4462	0.7111	0.0898	0.1998	0.8223	0.2785	0.3417	0.5980	0.7448
d 0 to 42												
ADG, lb	0.1629	0.0095	0.5932	0.2084	0.8000	0.3265	0.3404	0.5550	0.6127	0.7784	0.7569	0.4483
ADFI, lb	0.3007	0.0278	0.2756	0.2298	0.5188	0.9552	0.6133	0.3273	0.6331	0.2520	0.0905	0.0958
F/G	0.0813	0.0238	0.9105	0.5854	0.9360	0.3061	0.1858	0.9872	0.4609	0.6301	0.4324	0.8471

<sup>1</sup>A total of 288 nursery pigs (DNA Line 200 × 400; initially 11.8 lb BW) were used in a 42-d study with 4 pigs per pen and 8 replications per treatment.

<sup>2</sup>Phase 1 Experimental treatment diets were fed from d 0 to 7. Supplements were added to diets as seen on Table 1.

<sup>3</sup>Phase 2 Experimental treatment diets were fed from d 7 to 28. Supplements were added to diets as seen on Table 2.

## Effects of Increasing Salt Concentration for 15 to 22 lb Nursery Pigs

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### Summary

A total of 325 maternal line barrows (Line 200 × 400; DNA, Columbus, NE; initially 14.6 lb BW) were used in a 14-d growth trial to determine the optimal inclusion rate of dietary salt for growth performance of nursery pigs weighing approximately 15 to 22 lb. Upon entry of the nursery, pigs were allotted by BW and fed a common starter diet (6 lb/ton added salt and 25% dried whey) for 7 d after weaning. At d 7 after weaning, considered d 0 in the trial, pigs were allotted by pen weight and assigned to 1 of 5 dietary treatments. Treatments included a diet containing 10% dried whey with no added salt, or 4, 8, 12, and 16 lb/ton of added salt. A common Phase 3 diet, containing 7 lb/ton added salt, was then fed from d 14 to d 21.

From d 0 to 14, increasing salt increased (linear,  $P < 0.015$ ) ADG and ADFI. Feed efficiency improved (quadratic,  $P < 0.034$ ) as added salt increased from 0 to 12 lb with no further benefits observed thereafter. From d 14 to 21, when pigs were fed a common Phase 3 diet (7 lb/ton added salt), those previously fed no added salt had 20% greater ADG (linear,  $P < 0.013$ ) than those previously fed 4 to 16 lb added salt. The compensatory ADG observed from d 14 to 21 resulted in no overall differences in ADG, ADFI, or F/G from d 0 to 21. In conclusion, it appears that 12 lb/ton of added salt in a diet containing 10% dried whey optimizes ADG, ADFI, and F/G in 15 to 22 lb nursery pigs.

Key words: Cl, Na, nursery pig, salt

### Introduction

Sodium and chloride are key ions that are involved in metabolic processes and electrolyte balance in the body. Salt levels have been positively correlated to growth, feed

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efficiency, and feed intake (Mahan et al., 1996<sup>2</sup>;1999<sup>3</sup>). Monegue et al. (2011)<sup>4</sup> observed that pigs had a preference for a diet with added salt versus no added salt and that increasing added salt up to 16 lb/ton resulted in increased ADG and ADFI. Kerr et al. (1994)<sup>5</sup> reported that in Phase 2 diets formulated with 10% dried whey, 7 lb/ton of added salt resulted in improved ADG and F/G compared to treatments containing 0 and 3.5 lb/ton added salt. The NRC (2012)<sup>6</sup> requirement estimates for Na and Cl are 0.35 and 0.45%, respectively, for 15 to 25 lb pigs. Because dried whey typically contains 2 to 3% salt, most Phase 2 diets contain fixed amounts of 5 to 7 lb/ton added salt to try to meet the requirement estimate. However, despite the Na provided by dried whey and 7 lb/ton added salt, the Na concentration may be deficient in Phase 2 diets based on NRC requirement estimates (0.35% Na). Therefore, the objective of this study was to determine the dietary salt requirements for nursery pigs weighing 15 to 25 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS. Each pen was equipped with a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. A total of 325 maternal line barrows (Line 200 × 400; DNA, Columbus, NE) were used in a 14-d growth trial. Pigs were weaned at 21 d of age and placed into the nursery. Initially, pigs were randomly allotted to pens of 5 based on their initial BW. Pigs were fed a common diet for 7 d after weaning (Table 1). At d 7 after weaning, considered d 0 in the trial, pigs were randomly assigned to 1 of 5 dietary treatments with 13 replications per treatment. Dietary treatments were corn-soybean meal-based containing 10% dried whey and no added salt, or diets with either 4, 8, 12, or 16 lb/ton of added salt; this resulted in calculated dietary Na levels of 0.13, 0.21, 0.29, 0.37, and 0.45%, respectively. Pigs were then fed a common diet from d 14 to 21 (7 lb/ton added salt). Pens of pigs were weighed and feed disappearance was recorded on d 0, 7, 14, and 21 to determine ADG, ADFI, and F/G.

All experimental diets were fed in meal form and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. To create the intermediate diets, the low (no added salt) and high (16 lb/ton salt) treatment diets were manufactured and then blended at the mill. Experimental diets were achieved by replacing corn with salt.

<sup>2</sup> Mahan, D. C., E. A. Newton, and K. R. Cera. 1996. Effect of supplemental sodium chloride, sodium phosphate, or hydrochloric acid in starter pig diets containing dried whey. *J. Anim. Sci.* 74:1217-1222.

<sup>3</sup> Mahan, D. C., T. D. Wiseman, E. Weaver, and L. Russell. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. *J. Anim. Sci.* 77:3016-3021.

<sup>4</sup> Monegue, J.S., M.D. Lindemann, H.J. Monegue, and G.L. Cromwell. 2011. Growth performance and diet preference of nursery pigs fed varying levels of salt. *J. Anim. Sci.* 89 (E-Supplement 2):104.

<sup>5</sup> Kerr, C. A., R. D. Goodband, M. D. Tokach, J. L. Nelssen, S. S. Dritz, B. T. Richert, J. R. Bergstrom, and W. B. Nessmith. 1994. The effects of added salt in the Phase II starter pig diet. *Kansas Swine Industry Day Report of Progress* 717.

<sup>6</sup> NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Diet samples were taken from 8 feeders per dietary treatment and subsampled. Sub-samples were analyzed for DM, CP, Na, Cl, and salt (Ward Laboratories, Inc., Kearney, NE, Table 2).

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Linear and quadratic polynomials were used to evaluate increasing added salt. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ .

## Results and Discussion

Chemical analysis indicated that calculated values for Na and Cl were similar to analyzed values. Sodium ranged from 0.09 to 0.40% and Cl ranged from 0.23 to 0.72% (Table 2). Salt content ranged from 0.38 to 1.19% across treatment diets. This indicates that the 10% dried whey contributed approximately 7.6 lb salt to the diet.

From d 0 to 14, increasing added salt increased (linear,  $P < 0.015$ ) ADG and ADFI. Feed efficiency improved (quadratic,  $P < 0.034$ ) as added salt increased from 0 to 12 lb with no further benefits observed thereafter (Table 3). From d 7 to 14, increasing salt also increased (linear,  $P < 0.050$ ) ADG, ADFI, and d 14 BW. There was no effect on F/G.

From d 14 to 21, when all pigs were fed a common diet (7 lb salt), those previously fed no added salt had a 20% greater ADG and better F/G than those previously fed 4 to 16 lb added salt (linear,  $P < 0.013$ ). Because of the compensatory ADG observed during d 14 to 21, there were no overall (d 0 to 21) differences in ADG, ADFI, F/G, or final BW.

According to the chemical analysis, the diet with 12 lb/ton added salt contained 0.38% and 0.56%, Na and Cl, respectively (Table 2). The diet with 12 lb/ton added salt would be relatively similar in Na concentration to the NRC (2012)<sup>6</sup> estimate of 0.35%. Dietary Cl concentration was greater than the optimal level suggested by Mahan et al. (1999)<sup>3</sup> of 0.38% or the NRC (2012)<sup>7</sup> recommendation of 0.40%. Kerr et al. (1994)<sup>5</sup> added 3.5 or 7 lb/ton salt to diets containing 10% dried whey and tended to see a linear improvement in ADG and F/G. Monegue et al. (2011)<sup>4</sup> observed that increasing added salt up to approximately 10 lb/ton optimized performance; however, their diets were formulated with only added crystalline lactose, not dried whey as in our study.

In conclusion, 12 lb/ton of added salt, in a diet already containing 10% dried whey, appeared to optimize ADG and F/G in 15 to 22 lb nursery pigs. Further research should be conducted to see if the growth responses were due to an increase in Na or Cl, or both.

<sup>7</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

**Table 1. Diet composition (as-fed basis)**

Item	Common Phase 1 <sup>1</sup>	Experimental <sup>2</sup>	Common Phase 3 <sup>3</sup>
Ingredient %			
Corn	39.28	51.00	63.77
Soybean meal (48% CP)	17.65	29.60	32.86
Corn DDGS (6-9% oil)	5.00	-	-
Fish meal	4.50	-	-
Dried whey	25.00	10.00	-
HP 300 (Hamlet Protein)	2.50	5.00	-
Choice white grease	3.00	1.00	-
Monocalcium P (21% P)	0.40	1.05	1.10
Limestone	0.50	1.05	0.98
Salt	0.30	-	0.35
L-Lys-HCl	0.48	0.30	0.30
DL-Met	0.20	0.18	0.12
L-Thr	0.18	0.15	0.12
L-Trp	0.05	-	-
L-Val	0.10	-	-
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
HiPhos 2700	-	0.02	0.02
Zinc oxide	0.39	0.25	-
Choline chloride	0.04	-	-
Vitamin E, 20,000 IU	0.05	-	-
Total	100.00	100.00	100.00

*continued*

**Table 1. Diet composition (as-fed basis)**

Item	Common Phase 1 <sup>1</sup>	Experimental <sup>2</sup>	Common Phase 3 <sup>3</sup>
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.40	1.35	1.35
Ile:Lys	55	63	57
Leu:Lys	111	124	117
Met:Lys	36	35	30
Met and Cys:Lys	56	59	51
Thr:Lys	62	66	57
Tryp:Lys	19	19	17
Val:Lys	67	67	62
Total Lys, %	1.55	1.49	1.37
ME, kcal/lb	1,579	1,517	1,483
NE kcal/lb	1,191	1,053	1,072
SID Lys:ME, g/Mcal	4.02	4.04	4.13
CP, %	21.0	22.8	21.4
Ca, %	0.67	0.78	0.70
P, %	0.67	0.68	0.64
Available P, %	0.57	0.48	0.41
Na, %	0.39	0.13	0.18
Cl, %	0.78	0.40	0.49

<sup>1</sup>Common Phase 1 diet fed to all pigs from d 0 to 7 after weaning.

<sup>2</sup>Experimental diets were fed to pigs from d 7 to 21 after weaning. Corn was removed and replaced with salt to create the treatment diets. Treatment diets containing 0 and 16 lb/ton salt were manufactured and blended at the feed mill to create the intermediate levels of 4, 8, and 12 lb/ton.

<sup>3</sup>Common Phase 3 diet fed to all pigs from d 21 to 28 after weaning.

**Table 2. Chemical analysis of experimental diets (as-fed basis)<sup>1</sup>**

Item, %	Added salt, lb/ton				
	0	4	8	12	16
DM	90.13	89.94	90.08	90.14	90.93
CP	22.8	22.4	23.2	22.6	22.9
Na	0.09	0.17	0.23	0.38	0.40
Cl	0.23	0.37	0.46	0.56	0.72
Salt	0.38	0.62	0.76	0.92	1.19

<sup>1</sup>Multiple samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Ward Laboratories, Inc., Kearney, NE).

**Table 3. Effects of increasing salt for 15 to 22 lb nursery pigs<sup>1</sup>**

Item	Added salt, lb/ton <sup>2</sup>					SEM	<i>P</i> value	
	0	4	8	12	16		Linear	Quadratic
d 0 to 7 <sup>3</sup>								
ADG, lb	0.24	0.25	0.33	0.38	0.35	0.016	<0.001	0.106
ADFI, lb	0.43	0.39	0.44	0.46	0.46	0.019	0.026	0.396
F/G	1.78	1.57	1.33	1.24	1.30	0.047	<0.001	<0.001
d 7 to 14								
ADG, lb	0.62	0.70	0.70	0.75	0.76	0.042	0.004	0.478
ADFI, lb	0.93	0.96	0.97	0.98	1.02	0.036	0.050	0.867
F/G	1.54	1.57	1.41	1.33	1.36	0.125	0.117	0.913
d 0 to 14								
ADG, lb	0.43	0.48	0.52	0.56	0.56	0.023	<0.001	0.218
ADFI, lb	0.68	0.67	0.70	0.72	0.74	0.021	0.015	0.609
F/G	1.60	1.45	1.38	1.30	1.33	0.048	<0.001	0.034
d 14 to 21								
ADG, lb	0.95	0.78	0.80	0.84	0.74	0.054	0.013	0.318
ADFI, lb	1.23	1.15	1.21	1.21	1.17	0.054	0.701	0.911
F/G	1.31	1.51	1.57	1.48	1.64	0.065	0.002	0.313
d 0 to 21								
ADG, lb	0.60	0.58	0.61	0.65	0.62	0.024	0.169	0.918
ADFI, lb	0.86	0.83	0.87	0.88	0.88	0.025	0.259	0.768
F/G	1.44	1.45	1.44	1.36	1.44	0.030	0.286	0.528
BW, lb								
d 0	14.6	14.6	14.6	14.6	14.6	0.104	0.630	0.789
d 7	16.3	16.4	17.0	17.3	17.1	0.174	<0.001	0.107
d 14	20.7	21.3	21.9	22.5	22.5	0.330	<0.001	0.297
d 21	27.4	26.8	27.4	28.3	27.6	0.550	0.229	0.982

<sup>1</sup> A total of 325 maternal line barrows (Line 200 × 400; DNA, Columbus, NE) were used in a 14-d study with 5 pigs per pen and 13 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 7 d post-weaning, then placed on experimental diets.

<sup>2</sup> Treatment diets with 0 and 16 lb salt/ton were manufactured and blended at the feed mill to create the intermediate levels of 4, 8, and 12 lb salt/ton.

<sup>3</sup> Experimental diets were fed from d 0 to 14 and a common Phase 3 diet was fed from d 14 to 21.

## Evaluation of Added Sodium and Chloride for 15 to 24 lb Nursery Pigs

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### Summary

A total of 360 pigs (Line 241 × 600; DNA, Columbus, NE) were used in a 14-d growth trial to determine if the response to added dietary salt in nursery pigs (15 to 24 lb) was due to either the Na or Cl concentration in the diet. Upon entry to the nursery, pigs were allotted by BW and fed a common starter diet (0.33% Na and 0.76% Cl) for 7 d after weaning. On d 7 after weaning, considered d 0 in the trial, pens were assigned to 1 of 4 dietary treatments that were fed from d 0 to 14. The 4 experimental treatments included a 10% dried whey diet with 12 lb/ton added salt (0.37% Na and 0.75% Cl); or 3 diets with dried whey replaced by 7.2% lactose containing either: 7 lb/ton added salt (0.18% Na and 0.47% Cl); 15.5 lb/ton added salt (0.35% Na and 0.72% Cl); or 23 lb/ton sodium bicarbonate and 8 lb/ton potassium chloride (0.35% Na and 0.45% Cl), respectively. From d 0 to 14, pigs fed the 10% dried whey diet with 12 lb/ton added salt or the diet with lactose and 15.5 lb/ton added salt had improved ( $P < 0.05$ ) ADG compared to pigs fed the lactose diet with 7 lb/ton added salt, with pigs fed the lactose diet with 23 lb/ton sodium bicarbonate and 8 lb/ton potassium chloride intermediate. Pigs fed the 10% dried whey diet with 12 lb/ton added salt had greater ( $P < 0.05$ ) ADFI than those fed the lactose diet with 7 lb/ton added salt, with pigs fed the lactose diet with 15.5 lb/ton added salt and the lactose diet with 23 lb/ton sodium bicarbonate and 8 lb/ton potassium chloride intermediate. However, F/G tended to be poorest for pigs fed 10% dried whey compared with pigs fed 7.2% lactose and 15.5 lb/ton added salt, with others intermediate. In conclusion, diets should be formulated with enough added salt in order to meet NRC (2012)<sup>2</sup> recommendation of dietary Na concentration of 0.35%, which is higher in Na than many nursery diets for 15 to 25 lb pigs.

Key words: Cl, Na, nursery pig, salt

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<sup>2</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

## Introduction

Dietary salt concentrations have been positively correlated to growth, feed efficiency, and feed intake (Mahan et al., 1996;<sup>3</sup> 1999<sup>4</sup>). The NRC (2012)<sup>2</sup> requirement for Na and Cl are 0.35 and 0.45%, respectively, for 15 to 25 lb pigs. With diets containing spray-dried blood plasma, lactose, and added salt, Mahan et al. (1999)<sup>4</sup> noted improvement in F/G up to a dietary salt concentration of 0.40%. When Na and Cl were independently analyzed, Mahan et al. (1999)<sup>4</sup> reported that F/G and growth was positively affected up to a 0.45% Cl, but there were no advantages to increasing dietary Na concentration beyond 0.20%. Because 10% dried whey typically contains 2 to 3% salt, most Phase 2 nursery diets typically contain 5 to 7 lb/ton added salt, but the dietary Na concentrations of the diets may still be deficient according to NRC (2012)<sup>2</sup> recommendations. In a previous study, Shawk et al. (2016)<sup>5</sup> observed improvement in ADG, ADFI, and F/G up to 12 lb/ton added salt in Phase 2 diets containing 10% dried whey. The calculated analysis of this 12 lb/ton added salt diet had a Na concentration of 0.37% and a Cl concentration of 0.72%. This increase in performance observed by Shawk et al. (2016)<sup>5</sup> could have been a result of either the dietary Na concentration matching NRC (2012)<sup>2</sup> recommendations, the Cl concentration exceeding NRC (2012)<sup>2</sup> recommendations, or both. Thus, the purpose of this study was to determine if Na and Cl, concentration or their source had an effect on the growth of nursery pigs weighing 15 to 24 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen was equipped with a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. A total of 360 pigs (Line 241 × 600; DNA, Columbus, NE) were used in a 14-d growth trial. Pigs were weaned at approximately 21 d of age and placed into the nursery. Initially, pigs were randomly allotted to pens of 6 based on their initial BW. Pigs were fed a common diet (0.33 and 0.76% Na and Cl, respectively) for 7 d after weaning. On d 7 after weaning, considered d 0 in the trial, pens were randomly assigned to 1 of 4 dietary treatments with 15 replications per treatment. The 4 experimental treatments included a 10% dried whey diet with 12 lb/ton added salt (0.37% Na and 0.75% Cl); or 3 diets with dried whey replaced by 7.2% crystalline lactose with either: 7 lb/ton added salt (0.18% Na and 0.47% Cl); 15.5 lb/ton added salt (0.35% Na and 0.72% Cl); or 23 lb/ton sodium bicarbonate and 8 lb/ton potassium chloride (0.35% Na and 0.45% Cl; Table 1). Pens of pigs were weighed and feed disappearance was recorded on d 0, 7, and 14 to determine ADG, ADFI, and F/G.

<sup>3</sup> Mahan, D. C., E. A. Newton, and K. R. Cera. 1996. Effect of supplemental sodium chloride, sodium phosphate, or hydrochloric acid in starter pig diets containing dried whey. *J. Anim. Sci.* 74:1217-1222.

<sup>4</sup> Mahan, D. C., T. D. Wiseman, E. Weaver, and L. Russell. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. *J. Anim. Sci.* 77:3016-3021.

<sup>5</sup> D.J. Shawk, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, S.S. Dritz, J.C. Woodworth, H. E. Williams, and A. B. Clark. 2016. Effects of increasing salt concentration for 15 to 22 lb nursery pigs. Kansas Swine Industry Day, 2016, 17-118-J. Kansas Agricultural Experiment Station Research Reports, Volume 2, Issue 8.

All experimental diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Dietary treatments were corn-soybean meal-based and were fed in meal form. Corn was removed and replaced with an equal amount of either salt, potassium chloride, and/or sodium bicarbonate to create the treatment diets. Dried whey was replaced with crystalline lactose to create diets with the same lactose content, and all diets were formulated to the same NE concentration.

Diet samples were collected at the mill and subsampled. Subsamples were analyzed for DM, CP, Na, and Cl (Ward Laboratories, Inc., Kearney, NE, Table 2).

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and dietary treatment as the fixed effect. The main effects that were tested were the dietary Na and Cl concentration. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ .

## Results and Discussion

Chemical analysis indicated that the dietary Na concentration of the treatment diets was similar to formulated values, but the analyzed dietary Cl concentrations were slightly lower than formulated (Table 2). Sodium ranged from 0.18% to 0.37% and Cl ranged from 0.35% to 0.67%. The dried whey contained 0.79% Na and 1.45% Cl.

From d 0 to 14, pigs fed the 10% dried whey diet with 12 lb/ton added salt and those fed 7.2% lactose and 15.5 lb/ton added salt had greater ADG ( $P < 0.05$ ) than pigs fed the 7.2% lactose diet with 7 lb/ton added salt, with intermediate performance observed for pigs fed the lactose diet with 23 lb/ton sodium bicarbonate and 8 lb/ton potassium chloride. Pigs fed the whey diet with 12 lb/ton added salt had the highest ( $P < 0.05$ ) ADFI and pigs fed the lactose diet containing 7 lb/ton salt had the lowest, with the other treatments intermediate. There was a trend ( $P < 0.10$ ) for improved F/G of pigs fed 7.2% lactose and 15.5 lb/ton added salt compared with those fed 10% dried whey and 12 lb/ton added salt, with the others intermediate.

All treatment diets (0.37% Na), except the lactose diet with the 7 lb/ton added salt (0.18% Na), had a similar dietary Na concentration to NRC (2012)<sup>2</sup> recommendations and greater ADG and ADFI than the diet that was deficient in Na. Due to no significant difference in ADG, ADFI, and F/G between the 10% dried whey diet with 12 lb/ton added salt and the lactose diet with 15.5 lb/ton added salt, results would suggest that the source of the salt in the diet (dried whey plus salt vs only salt) had no effect on overall performance. Results of this study would indicate the additional performance observed by Shawk et al. (2016)<sup>5</sup> was a result of dietary Na concentration meeting NRC (2012)<sup>2</sup> recommendations.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	12 lb/ton added salt, dried whey	7 lb/ton added salt, lactose	15.5 lb/ton added salt, lactose	KCl and NaHCO <sub>3</sub> , lactose
Ingredient %				
Corn	50.36	50.47	49.76	48.59
Soybean meal (48% CP)	29.65	29.67	29.66	29.65
Lactose	---	7.20	7.20	7.20
Dried whey	10.00	---	---	---
HP 300 <sup>2</sup>	5.00	7.75	7.80	7.88
Choice white grease	1.00	0.90	1.15	1.55
Monocalcium P (21% P)	1.05	1.33	1.33	1.15
Limestone	1.05	1.05	1.05	1.15
Potassium chloride	---	---	---	0.40
Sodium bicarbonate	---	---	---	1.15
Salt	0.60	0.35	0.78	---
Zinc oxide	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25
Phytase <sup>3</sup>	0.02	0.02	0.02	0.02
L-Lys-HCl	0.30	0.30	0.30	0.30
DL-Met	0.18	0.17	0.17	0.17
L-Thr	0.15	0.16	0.16	0.16
Total	100	100	100	100

*continued*

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	12 lb/ton added salt, dried whey	7 lb/ton added salt, lactose	15.5 lb/ton added salt, lactose	KCl and NaHCO <sub>3</sub> , lactose
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	1.35	1.35	1.35	1.35
Ile:Lys	63	63	63	63
Leu:Lys	123	123	123	122
Met:Lys	35	35	35	34
Met and Cys:Lys	58	58	58	57
Thr:Lys	66	65	65	65
Tryp:Lys	19	19	19	19
Val:Lys	67	68	68	68
Total Lys, %	1.49	1.49	1.49	1.49
NE kcal/lb	1,110	1,110	1,110	1,110
SID Lys:ME, g/Mcal	4.06	4.05	4.05	4.06
CP, %	22.8	23.2	23.1	23.1
Ca, %	0.78	0.78	0.78	0.78
P, %	0.68	0.69	0.69	0.65
Available P, %	0.48	0.48	0.48	0.48
Na, %	0.37	0.18	0.35	0.35
Cl, %	0.75	0.47	0.72	0.45
K, %	1.14	1.02	1.01	1.22
Dietary electrolyte balance	240	205	207	337

<sup>1</sup> Experimental diets were fed from d 7 to 21 after weaning.

<sup>2</sup> Hamlet Protein, Findlay, OH.

<sup>3</sup> HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

**Table 2. Chemical analysis of experimental diets (as-fed basis)<sup>1</sup>**

Item, %	12 lb/ton added salt, dried whey	7 lb/ton added salt, lactose	15.5 lb/ton added salt, lactose	KCl and NaHCO <sub>3</sub> , lactose
DM	89.68	88.82	89.79	90.2
CP	22.5	22.3	22.4	22.1
Na	0.37	0.18	0.37	0.37
Cl	0.67	0.36	0.60	0.35

<sup>1</sup> Multiple samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Ward Laboratories, Inc., Kearney, NE).

**Table 3. Effects of increasing Na and Cl for 15 to 24 lb nursery pigs <sup>1</sup>**

Item	Experimental diets				SEM	Probability, <i>P</i> <
	12 lb/ton added salt, dried whey	7 lb/ton added salt, lactose	15.5 lb/ton added salt, lactose	KCl and NaHCO <sub>3</sub> , lactose		
d 0 to 14						
ADG, lb	0.62 <sup>a</sup>	0.55 <sup>b</sup>	0.63 <sup>a</sup>	0.60 <sup>ab</sup>	0.021	0.038
ADFI, lb	0.98 <sup>a</sup>	0.86 <sup>c</sup>	0.94 <sup>ab</sup>	0.90 <sup>bc</sup>	0.025	0.004
F/G	1.59 <sup>x</sup>	1.56 <sup>xy</sup>	1.50 <sup>y</sup>	1.52 <sup>xy</sup>	0.031	0.086
BW, lb						
d 0	15.3	15.3	15.3	15.3	0.13	0.999
d 7	18.3	17.8	18.3	18.0	0.23	0.374
d 14	23.9 <sup>xy</sup>	23.0 <sup>x</sup>	24.2 <sup>y</sup>	23.6 <sup>xy</sup>	0.34	0.080

<sup>ab</sup> Means with common superscripts differ  $P < 0.05$ .

<sup>xy</sup> Means with common superscripts differ  $P < 0.10$ .

<sup>1</sup> A total of 360 barrows (Line 241 × 600; DNA, Columbus, NE) were used in a 14-d study with 6 pigs per pen and 15 pens per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 7 d post-weaning, then placed on experimental diets.

## Comparing the Effects of Butyric Acid Source and Level on Growth Performance of Nursery Pigs<sup>1</sup>

*K.M. Gourley, J.C. Woodworth, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, and S.S. Dritz<sup>2</sup>*

### Summary

A total of 398 pigs (PIC 19 × 1050 or PIC 3 × C29, initially  $13.56 \pm 0.02$  lb) were used in a 42-d growth study to compare the effects of increasing two different sources of encapsulated butyric acid on growth performance of nursery pigs fed meal diets. Dietary treatments were arranged as a  $2 \times 2 + 1$  factorial with main effects of butyric acid source (ButiPEARL vs. ButiPEARLZ; Kemin Industries, Des Moines, IA) and level (low (1 or 1.38 lb/ton) vs. high (2 or 2.76 lb/ton) respectively) plus a control diet without any butyric acid. The inclusion rates of each product were established such that the same amount of butyric acid was contributed from each source for the low or high levels, respectively. Experimental diets were fed in three phases from d 0 to 7, 7 to 21, and 21 to 42. Pens of pigs (6 barrows and 4 gilts) were balanced by initial BW and randomly allotted to treatments, with 8 replications (pens) per treatment. From d 0 to 7, a source × level interaction ( $P < 0.05$ ) was observed for ADG, ADFI, and F/G, with pigs fed diets containing ButiPEARL having improved performance at the low inclusion, but with those fed high butyric acid not different from the control. However, pigs fed ButiPEARLZ had poorer growth performance at the low level, with the high level having performance similar to the control. In Phase 2 (d 7 to 21), ADG and ADFI were not influenced by butyric acid source or level, but an interaction ( $P = 0.001$ ) was observed for F/G as pigs fed ButiPEARL had poorer F/G as level increased; whereas pigs fed increasing ButiPEARLZ had improved F/G. For Phase 3 (d 21 to 42), increasing either butyric acid source tended ( $P = 0.060$ ) to decrease ADG. Overall (d 0 to 42), butyric acid source or level did not affect ADG, ADFI or F/G. In conclusion, this study showed that pigs fed low ButiPEARL in Phase 1 (d 0 to 7) had improved growth performance compared to other treatments with only minor treatment effects observed thereafter. More research is warranted to determine if the butyric acid sources used in this experiment would elicit different responses in pelleted nursery diets.

Key words: butyric acid, growth, nursery pigs

<sup>1</sup> Appreciation is expressed to Kemin Industries, (Des Moines, IA) for financial support of this experiment and to Julie Salyer of Kalmbach Feeds, Upper Sandusky, OH, for assistance in conducting this experiment.

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## Introduction

With evolving research on feed additives in the nursery, there is an interest to determine which products are effective at increasing growth and efficiency of weaned pigs. One such feed additive is butyric acid, which is a short chain fatty acid that is used by the gastrointestinal tract to promote growth of the intestinal epithelium in monogastric animals. Due to the pungent odors commonly associated with it, and to make it easier to handle in feed mills, it is often encapsulated. Encapsulation is a process where a liquid can be captured inside of a shell through a spray freezing technology. The shell, which consists of a fat matrix, ensures that the ingredient is not digested immediately and can be released in the intestinal tract. Kemin Industries (Des Moines, IA) manufactures and markets an encapsulated butyric acid product called ButiPEARL. Recently, the company has developed a next generation encapsulated butyric acid product called ButiPEARLZ, which is suggested to have differing butyric acid release rates from the encapsulation matrix, that might impact growth performance. No data are available to compare the two products. Therefore, the objective of this study was designed to compare the two sources and levels of butyric acid, in meal diets on the growth performance of nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. To better reflect the increased challenge of rearing pigs in a commercial environment, rooms were not washed before the experiment to increase bacteria load.

A total of 398 pigs (PIC 19 × 1050 or PIC 3 × C29, initially  $13.56 \pm 0.02$  lb) were used in a 42-d growth study. Pens of pigs (6 barrows and 4 gilts) were balanced by initial BW and randomly allotted to treatments with 8 replications (pens) per treatment. The 5 dietary treatments were initiated immediately after weaning and were arranged as a 2 × 2 + 1 factorial with a control diet, the control + ButiPEARL (1 or 2 lb/ton; Kemin Industries, Des Moines, IA), and control + ButiPEARLZ (1.38 or 2.76 lb/ton). The inclusion rates of each product were established such that each source contributed the same amount of butyric acid for the low or high levels, respectively. Experimental diets (Tables 1 and 2) were fed in 3 phases from d 0 to 7, 7 to 21, and 21 to 42. Feed was manufactured at the Kalmbach Feeds feed mill and fed in meal form. Multiple feed samples were collected at the feeder during each phase and analyzed for CP, Ca and P (Ward Laboratories, Inc., Kearney, NE). Pig weight and feed disappearance were measured on d 0, 7, 14, 21, 28, 35, and 42 to determine ADG, ADFI, and F/G.

Data were analyzed using the PROC MIXED procedures of SAS (SAS Institute Inc., Cary, NC) in a randomized design with pen serving as the experimental unit. The main effects of butyric acid source and level, and their interactions, were tested with results considered significant at  $P \leq 0.05$  and a trend at  $P \leq 0.10$ .

## Results and Discussion

During Phase 1 (d 0 to 7), a source  $\times$  level interaction ( $P < 0.05$ ) was observed for ADG, ADFI, and F/G (Table 3). This was the result of pigs fed diets containing ButiPEARL having improved performance at the low inclusion level, with the high level no different than control; however, pigs fed ButiPEARLZ had poorer performance at the low level, with the high level having performance similar to the control (Table 3). Also, pigs fed diets containing ButiPEARL had improved ( $P \leq 0.005$ ) ADG and ADFI and tended to have improved ( $P = 0.083$ ) F/G compared to those fed ButiPEARLZ.

During Phase 2 (d 7 to 21), an interaction ( $P = 0.001$ ) was observed for F/G, with pigs fed increasing ButiPEARL having improved F/G; whereas pigs fed increasing ButiPEARLZ having poorer F/G. No main effects of source or level were observed. In Phase 3 (d 21 to 42), ADG tended to be greater ( $P = 0.060$ ) for pigs fed either low dietary butyric acid levels, than for those fed the high levels. Overall (d 0 to 42), there were no differences observed among treatments.

Previous research demonstrated that *n*-butyrate is the main energy substrate for colonocytes, where 75% of oxygen consumed by colonocytes is from metabolism of *n*-butyrate (Roediger, 1980<sup>3</sup>). Furthermore, when diets included tributyrin (a compound composed of butyric acid and glycerol) and were fed with lactic acid, this increased the mucosal thickness and villus length in the cecum (Piva et al., 2002<sup>4</sup>).

In summary, this study shows that low levels of ButiPEARL will elicit improved growth performance of pigs the first week after weaning. More research is warranted to determine if the butyric acid sources used in this experiment would elicit different responses in pelleted nursery diets.

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<sup>3</sup> Roediger, W. E. 1980. Role of anaerobic bacteria in the metabolic welfare of the colic mucosa in man. *Gut*. 21:793-798.

<sup>4</sup> Piva, A. A., Prandini, L. Fiorentini, M. Morlacchini, F. Galvano, and J.B. Luchansky. 2002. Tributyrin and lactic acid synergistically enhanced the trophic status of the intestinal mucosa and reduced histamine levels in the gut of nursery pigs. *J. Anim. Sci.* 80:670-680.

**Table 1. Diet composition (as-fed basis)**

Ingredients, %	Phase 1 <sup>1</sup>	Phase 2	Phase 3
Corn	38.50	50.88	62.05
Soybean meal	21.50	30.10	32.23
Dried whey	20.90	10.15	---
Cheese plus <sup>2</sup>	7.30	---	---
Spray dried plasma	4.00	---	---
Fish meal	2.50	3.25	---
Tallow	2.00	2.00	2.00
Limestone	1.02	1.03	1.08
Monocalcium phosphate, 21% P	0.91	1.07	0.83
Salt	0.25	0.25	0.50
L-Lysine HCl	0.19	0.30	0.34
DL-Methionine	0.17	0.17	0.15
Threonine	0.09	0.11	0.12
Copper sulfate	0.09	0.09	0.09
Zinc oxide	0.26	0.26	0.26
Trace mineral premix	0.09	0.09	0.09
Selenium 0.06%	0.02	0.02	0.02
Vitamin premix	0.05	0.05	0.05
Biotin 100 mg/lb	0.08	0.08	0.08
K-Vitamin E-20,0	0.06	0.06	0.06
Choline chloride, 70%	0.05	0.05	0.05
Quantum Blue <sup>3</sup>	---	---	0.01
ButiPEARL <sup>4</sup>	---	---	---
ButiPEARLZ <sup>5</sup>	---	---	---
Total	100.0	100.0	100.0

*continued*

**Table 1. Diet composition (as-fed basis)**

Ingredients, %	Phase 1 <sup>1</sup>	Phase 2	Phase 3
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.52	1.35	1.25
Met:lys	34	37	36
Met and cys:lys	58	58	58
Thr:lys	65	65	65
Trp:lys	18	18	18
Val:lys	68	67	68
Total lys, %	1.68	1.50	1.25
ME, kcal/lb	1,587	1,534	1,530
CP, %	23.2	22.0	20.7
Ca, %	0.90	0.90	0.85
P, %	0.77	0.73	0.71
Available P, %	0.55	0.45	0.40

<sup>1</sup>Phase 1, 2, and 3 diets were fed from d 0 to 7, 7 to 21 and 21 to 42, respectively.

<sup>2</sup>Cheese Plus (International Ingredient Corporation, St. Louis, MO).

<sup>3</sup>Quantum Blue (AB Vista Americas, Plantation, FL) provided 227 FTU/lb of diet, with a release of 0.13% available P.

<sup>4</sup>ButiPEARL (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included in the diet at the expense of corn at low (1.0 lb/ton) or high (2.0 lb/ton) levels.

<sup>5</sup>ButiPEARLZ (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included in the diet at the expense of corn at low (1.38 lb/ton) or high (2.76 lb/ton) levels.

**Table 2. Chemical analysis of diets (as-fed basis)<sup>1,2</sup>**

Item,%	Phase 1					Phase 2					Phase 3				
	Control	ButiPEARL <sup>3</sup>		ButiPEARLZ <sup>4</sup>		Control	ButiPEARL		ButiPEARLZ		Control	ButiPEARL		ButiPEARLZ	
		Low	High	Low	High		Low	High	Low	High		Low	High	Low	High
DM	90.01	90.68	90.39	90.94	90.81	89.28	89.60	89.29	89.14	89.81	87.95	87.82	88.36	87.94	88.06
CP	22.4	22.9	22.6	23.3	23.1	23.1	19.9	21.2	22.0	21.9	19.9	19.8	18.4	19.5	18.4
Crude fiber	1.5	1.3	1.8	2.2	2.2	1.8	3.3	2.3	2.1	2.7	2.2	3.5	2.6	2.2	2.5
Ca	0.91	0.98	1.03	1.27	1.11	0.92	1.25	1.03	0.96	0.91	0.71	0.81	0.81	0.66	0.74
P	0.77	0.79	0.79	0.81	0.84	0.71	0.76	0.75	0.69	0.70	0.56	0.58	0.55	0.53	0.51
Ash	7.56	7.07	7.12	7.70	7.27	6.08	7.10	6.29	6.21	5.89	5.10	5.17	5.17	5.02	4.95
Ether extract	5.9	5.8	5.6	5.6	5.8	4.9	4.3	4.6	4.5	4.8	4.2	5.1	4.7	4.6	4.3

<sup>1</sup>Phase 1, 2, and 3 diets were fed from d 0 to 7, 7 to 21, and 21 to 42, respectively.

<sup>2</sup>Values represent a subsample from a composite sample collected at multiple feeders per treatment.

<sup>3</sup>ButiPEARL (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included in the diet at low (1.0 lb/ton) or high (2.0 lb/ton) levels.

<sup>4</sup>ButiPEARLZ (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included in the diet at low (1.38 lb/ton) or high (2.76 lb/ton) levels.

**Table 3. Effects of butyric acid source and level on performance of nursery pigs fed meal diets<sup>1</sup>**

Item	ButiPEARL <sup>2</sup>			ButiPEARLZ <sup>3</sup>		SEM	Probability, <i>P</i> <		
	Control	Low	High	Low	High		Source × level	Source	Level
BW, lb									
d 0	13.58	13.58	13.57	13.55	13.56	0.025	0.616	0.505	0.911
d 7	15.24 <sup>b</sup>	15.83 <sup>a</sup>	15.23 <sup>b</sup>	14.94 <sup>b</sup>	15.24 <sup>b</sup>	0.135	0.002	0.002	0.279
d 21	26.91	25.25	25.15	24.66	24.85	0.403	0.737	0.308	0.914
d 28	34.89	34.38	33.20	32.94	32.79	0.557	0.367	0.112	0.252
d 42	58.16	57.56	55.79	55.44	55.18	0.846	0.377	0.118	0.242
d 0 to 7									
ADG, lb	0.24 <sup>b</sup>	0.31 <sup>a</sup>	0.24 <sup>b</sup>	0.20 <sup>b</sup>	0.24 <sup>b</sup>	0.017	0.002	0.003	0.413
ADFI, lb	0.31 <sup>b</sup>	0.37 <sup>a</sup>	0.32 <sup>b</sup>	0.29 <sup>b</sup>	0.31 <sup>b</sup>	0.014	0.020	0.002	0.430
F/G	1.31 <sup>a,b</sup>	1.21 <sup>b</sup>	1.38 <sup>a,b</sup>	1.48 <sup>a</sup>	1.34 <sup>a,b</sup>	0.062	0.018	0.083	0.822
d 7 to 21									
ADG, lb	0.83	0.67	0.71	0.69	0.69	0.027	0.425	0.986	0.615
ADFI, lb	0.95	0.88	0.87	0.85	0.88	0.029	0.459	0.708	0.871
F/G	1.14 <sup>c</sup>	1.32 <sup>a</sup>	1.22 <sup>b</sup>	1.23 <sup>b</sup>	1.28 <sup>a,b</sup>	0.022	0.001	0.404	0.344
d 21 to 42									
ADG, lb	1.49	1.53	1.46	1.47	1.43	0.026	0.488	0.101	0.060
ADFI, lb	2.22	2.21	2.15	2.12	2.10	0.044	0.694	0.126	0.387
F/G	1.49	1.45	1.48	1.45	1.46	0.015	0.677	0.759	0.109
d 0 to 42									
ADG, lb	1.06	1.04	1.01	1.00	0.98	0.020	0.694	0.130	0.260
ADFI, lb	1.48	1.45	1.42	1.39	1.39	0.030	0.581	0.135	0.532
F/G	1.39	1.40	1.41	1.40	1.41	0.012	0.621	0.799	0.264

<sup>1</sup>A total of 398 pigs (PIC 19 × 1050 or PIC 3 × C29, 13.56 ± 0.02 lb) were used in a 42-d growth trial with 10 pigs per pen and 8 replications per treatment.

<sup>2</sup>ButiPEARL (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included at low (1.0 lb/ton) or high (2.0 lb/ton) levels.

<sup>3</sup>ButiPEARLZ (Kemin Industries Inc., Des Moines, IA) encapsulated butyric acid was included at low (1.38 lb/ton) or high (2.76 lb/ton) levels.

## Determining the Phosphorus Release for Natuphos E 5,000 G Phytase for Nursery Pigs<sup>1</sup>

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and R.D. Goodband*

### Summary

A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth trial to determine the available P (aP) release curve for a novel phytase source (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ). Pigs were randomly allotted to pens at weaning. On d 0 of the experiment (d 18 after weaning), pens were allotted in a randomized complete block design to 1 of 8 treatments. There were 4 pigs per pen and 9 pens per treatment. Pigs were fed a corn-soybean meal-based diet formulated to 1.25% standardized ileal digestible (SID) lysine. Ten 1-ton batches of basal feed (0.12% aP) were manufactured and subsequently divided to be the major portion of experimental diet manufacturing. Experimental diets were formulated to contain increasing aP supplied by either an inorganic source (0.12, 0.18, and 0.24% aP from monocalcium P) or from increased phytase (150, 250, 500, 750, and 1,000 FTU/kg). Diets were analyzed for phytase using the AOAC method and actual analyzed concentrations were 263, 397, 618, 1,100, and 1,350 FTU/kg, respectively. On d 21 of the study, one pig per pen was euthanized and the right fibula was collected for bone ash and percentage bone ash calculations. From d 0 to 21, increasing P from inorganic P or increasing phytase resulted in improved (linear,  $P < 0.01$ ) ADG, F/G and ending BW. Bone ash weight and percentage bone ash increased (linear,  $P < 0.01$ ) with increasing inorganic P or phytase. When formulated phytase values and percentage bone ash are used as the response variables, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 G phytase can be predicted by the equation: aP release =  $0.000212 \times \text{FTU/kg phytase}$ .

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

### Introduction

Phosphorus is an important macro mineral in swine nutrition. Along with calcium and vitamin D, it contributes to bone development. Most swine diets are formulated with cereal grains and oilseed, which contain P in the form of phytic acid. Monogastrics do not produce the enzyme needed to cleave the phosphates from the phytic acid for

<sup>1</sup> Appreciation is expressed to BASF Corporation, Florham Park, NJ, for partial funding of this project.

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absorption. As a result, a phytase enzyme is commonly added to swine diets to make P more available for animal use. This allows for reduced dietary inclusion of P from inorganic P sources, and results in reduced P excretion by the pig.

There are several phytase sources commercially available for swine producers to utilize, and as new generations of these products become available, updated P release values need to be determined. While some phytase products have already undergone evaluation to determine their unique release curve, other newer products have not been thoroughly tested.

Therefore, the objective for this trial was to evaluate the effects of a second generation phytase (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ) source on nursery pig performance and bone ash to develop an aP release curve.

## Procedures

The Kansas State Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The nursery barn was environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth trial. Pigs were initially weaned and randomly allotted to pens and fed a common diet. After 14 d post-weaning, pens of pigs were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 4 pigs per pen (2 barrows and 2 gilts) and 9 replications (pens) per dietary treatment. The dietary treatments consisted of 3 treatments (0.12, 0.18, or 0.24% aP) of increasing inorganic P, provided by monocalcium P, or 5 treatments consisting of added phytase (150, 250, 500, 750, or 1,000 FTU/kg) with the phytase added to the 0.12% aP inorganic P diet. Prior to the beginning of the 21-d study (d 15 to 18 post-weaning), all pigs were fed the negative control diet (0.12% aP) for a 4-d pre-test period.

Dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Ingredients containing Ca or P were analyzed (Ca, P) prior to manufacturing the diets in order to determine nutrient loading values used for formulation (Table 1). Phytase premix was also analyzed to determine inclusion rate in the experimental diets and contained 5,111,000 FTU/kg.

All dietary treatments were derived from 10, 1-ton basal batches (Table 2). After manufacturing, each basal batch was bagged off into 10 separate tons. For each experimental diet, a subset of bags (50 lb each) from the basal diet was added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 3). During bagging of experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, 30th, and 35th bags, and these samples were pooled and used for phytase and nutrient analysis.

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for proximate analysis, including

CP, Ca, and P. In addition, one sample was sent to another commercial feed laboratory (Eurofins Scientific Inc., Des Moines, IA) for complete diet phytase analysis (AOAC; method 2000.12).

During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G. On d 21 of the study, 1 pig per pen was euthanized via captive bolt. Pigs selected were the median weight gilt in each pen. The right fibula was removed from euthanized pigs to determine percentage ash bone criteria. Once collected, all fibulas were stored at -4°F. For processing of fibulas for bone ash, cartilage caps were removed, and bones were boiled for 60 min. Adhering tissue was removed and bones were dried at 221°F for 7 d. Then dried fibulas were ashed in a muffle furnace at 1,112°F for 24 h to determine total ash weight and percentage bone ash.

### *Data Analysis*

Studentized residuals were evaluated for pen means or individual bone ash measurements to ensure data met the assumption of normal distribution. One pig had a bone ash weight and percentage bone ash greater than 3 SD from the mean and was removed from bone ash analysis, but the pen data were retained for the evaluation of growth data.

Data were analyzed as a randomized complete block design with pen as the experimental unit. An initial base model was evaluated using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment was considered the fixed effect and linear and quadratic contrasts were evaluated within increasing inorganic P or phytase doses. Contrast coefficients for phytase doses were adjusted to account for the unequal spacing.

For pens fed inorganic phosphorus diets, the marginal intake of aP per day was calculated for each pen. The calculation was: dietary aP% minus 0.12% (the aP in the base diet) multiplied by ADFI. Subsequently, a standard curve was developed for each response criteria using marginal aP release as the predictor variable. The equation for the standard curve was then used to calculate aP release for each pen fed the different phytase dosages based on the observed value for each response criteria. This value was then converted to a marginal aP% using the pen ADFI.

Mixed model ANOVA with weight block as a random effect was then performed to evaluate aP release as a function of the phytase dosage using linear and quadratic contrasts. Next, mixed model regression was performed to predict aP release as a function of phytase dosage assuming an intercept of no aP release for the control diet without phytase.

Results were considered to be significant with  $P$ -values  $\leq 0.05$  and were considered marginally significant with  $P$ -values  $\leq 0.10$ .

### **Results and Discussion**

Crude protein and P of the experimental diets were similar to those expected from diet formulation. There was some variation in Ca analysis, which is similar to what we have observed in analysis of other experimental diets. The level of phytase analyzed slightly higher than expected across all diets (Table 3). This was unexpected due to the use of

the analyzed phytase level for dietary formulation. Analysis of phytase in final diets is much more difficult and variable than analysis of phytase in concentrated products, which may contribute to the higher analyzed values. Nevertheless, the phytase levels increase in a stepwise fashion as would be expected.

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear,  $P < 0.01$ , Table 4) ADG, ending BW and F/G. Additionally, pigs fed increasing phytase had improved (linear,  $P < 0.01$ ) ADG, ending BW, and F/G.

For bone composition, bone ash weights were increased (linear,  $P < 0.01$ ) for pigs fed either increasing inorganic P or phytase. As a result, percentage bone ash values increased (linear,  $P < 0.01$ ) for pigs fed inorganic P or phytase.

Percentage aP released from this phytase source varied depending on the response criteria (Table 5). As phytase concentrations increased, calculated aP increased in a linear ( $P < 0.01$ ) fashion to the highest phytase dose. The greatest aP release was calculated with percentage bone ash as the response criteria. Based on the linear response (Figure 2) for aP release associated with percentage bone ash, it appears the associated prediction equation (Table 6) will very closely predict the aP release. Release values for performance criteria (ADG and F/G) were lower than the release values for percentage bone ash. This might be a result of the Ca level used in the basal diets. Recent research indicates that high Ca to P ratios increase percentage bone ash, but may impair feed intake and growth rate.

Overall, this study has provided an aP release curve that can be used to value Natuphos E 5,000 phytase as a source of aP in nursery diets when included at levels between 150 and 1,000 FTU/kg. Available P release of percentage bone ash for up to 1,000 FTU/kg of Natuphos E 5,000 can be predicted by the equation: aP release =  $0.000212 \times \text{FTU/kg phytase}$ .

**Table 1. Analyzed ingredient composition (as-fed basis)<sup>1</sup>**

Ingredient	Analyzed value, %	
	Ca	P
Corn	0.03	0.29
Soybean meal	0.36	0.71
Limestone	29.25	0.14
Monocalcium P	17.37	20.85
Vitamin premix	17.15	0.04
Trace mineral premix	27.65	0.02

<sup>1</sup>Ingredient samples were pooled and analysis was performed by two commercial laboratories (Ward Laboratories, Kearney, NE, and Cumberland Valley Analytical Services, Hagerstown, MD).

**Table 2. Composition of basal batch (as-fed basis)<sup>1</sup>**

Item	
Ingredient, %	
Corn	63.67
Soybean meal	33.85
Monocalcium P	0.20
Limestone	1.04
Sodium chloride	0.35
L-Lys-HCL	0.30
DL-Met	0.12
L-Thr	0.12
Trace mineral premix	0.15
Vitamin premix	0.25
Calculated analysis	
SID <sup>2</sup> Lys, %	1.25
Total Lys, %	1.40
SID amino acid ratios	
Ile:Lys	63
Leu:Lys	129
Met:Lys	33
Met and Cys:Lys	57
Thr:Lys	63
Trp:Lys	18.7
Val:Lys	69
CP, %	21.8
NE, kcal/lb	1,100
SID Lys:ME, g/Mcal	3.78
Ca, %	0.64
P, %	0.54
Available P, %	0.12
STTD P, %	0.36

<sup>1</sup>The basal batch was used as the major ingredient within each experimental diet.

<sup>2</sup>Standardized ileal digestible.

**Table 3. Ingredient composition of experimental diets (as-fed basis)**

Ingredient, %	Experimental diet							
	Inorganic P			Phytase <sup>1</sup>				
	0.12%	0.18%	0.24%	150	250	500	750	1,000
Basal mix	99.01	99.01	99.01	99.01	99.01	99.01	99.01	99.01
Limestone	0.25	0.13	---	0.25	0.25	0.25	0.25	0.25
Monocalcium P	---	0.27	0.54	---	---	---	---	---
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sand <sup>2</sup>	0.34	0.20	0.05	0.34	0.34	0.33	0.33	0.32
Phytase	---	---	---	0.003	0.005	0.009	0.014	0.019
Calculated analysis								
CP, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
P, %	0.54	0.60	0.66	0.54	0.54	0.54	0.54	0.54
Phytase, FTU/kg	---	---	---	150	250	500	750	1000
Ca:P ratio	1.35	1.22	1.11	1.35	1.35	1.35	1.35	1.35
Analyzed composition								
CP, %	21.5	19.8	22.0	21.4	22.2	22.9	22.1	23.1
Ca, %	0.89	0.81	0.68	0.86	0.89	0.73	0.78	0.58
P, %	0.49	0.55	0.63	0.48	0.48	0.47	0.45	0.47
Phytase, FTU/kg	95	< 60	< 60	263	397	618	1100	1350
Ca:P ratio	1.81	1.48	1.08	1.81	1.85	1.55	1.72	1.23

<sup>1</sup>Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ). Phytase premix was analyzed for phytase level, and it contained 5,111,000 FTU/kg.

<sup>2</sup>Sand was used equalize inclusion rates of experimental ingredients.

<sup>3</sup> Phytase premix was analyzed for phytase level, and it contained 5,111,000 FTU/kg.

**Table 4. Effects of increasing aP from inorganic P or Natuphos E 5,000 G on nursery pig growth performance and bone ash values<sup>1</sup>**

Item	Inorganic P, % aP <sup>2</sup>				Phytase, FTU/kg <sup>3</sup>				SEM	Inorganic P		Phytase	
	0.12	0.18	0.24	150	250	500	750	1,000		Linear	Quadratic	Linear	Quadratic
BW, lb													
d 0	24.7	24.6	24.6	24.0	24.0	24.3	24.5	24.7	0.43	0.724	0.975	0.126	0.133
d 21	44.65	48.86	51.65	46.98	47.66	47.51	49.51	51.28	0.84	<0.001	0.478	<0.001	0.906
d 0 to 21													
ADG, lb	0.96	1.18	1.29	1.08	1.08	1.10	1.19	1.27	0.03	<0.001	0.111	<0.001	0.666
ADFI, lb	1.89	2.06	2.16	2.02	1.99	1.97	2.13	2.14	0.05	<0.001	0.517	<0.001	0.959
F/G	1.98	1.75	1.68	1.89	1.81	1.79	1.79	1.70	0.03	<0.001	0.050	<0.001	0.352
Bone ash weight, g <sup>4</sup>	0.678	0.850	0.856	0.713	0.666	0.769	0.819	0.936	0.041	0.003	0.103	0.001	0.194
Bone ash, % <sup>4</sup>	38.11	41.23	42.05	38.67	39.65	41.36	43.21	45.59	1.010	0.005	0.332	0.001	0.614

<sup>1</sup>A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth study evaluating the effects of increasing available P from inorganic P or from a novel phytase source.

<sup>2</sup>Inorganic P was added to the diet by increasing monocalcium P.

<sup>3</sup>Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ).

<sup>4</sup>One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

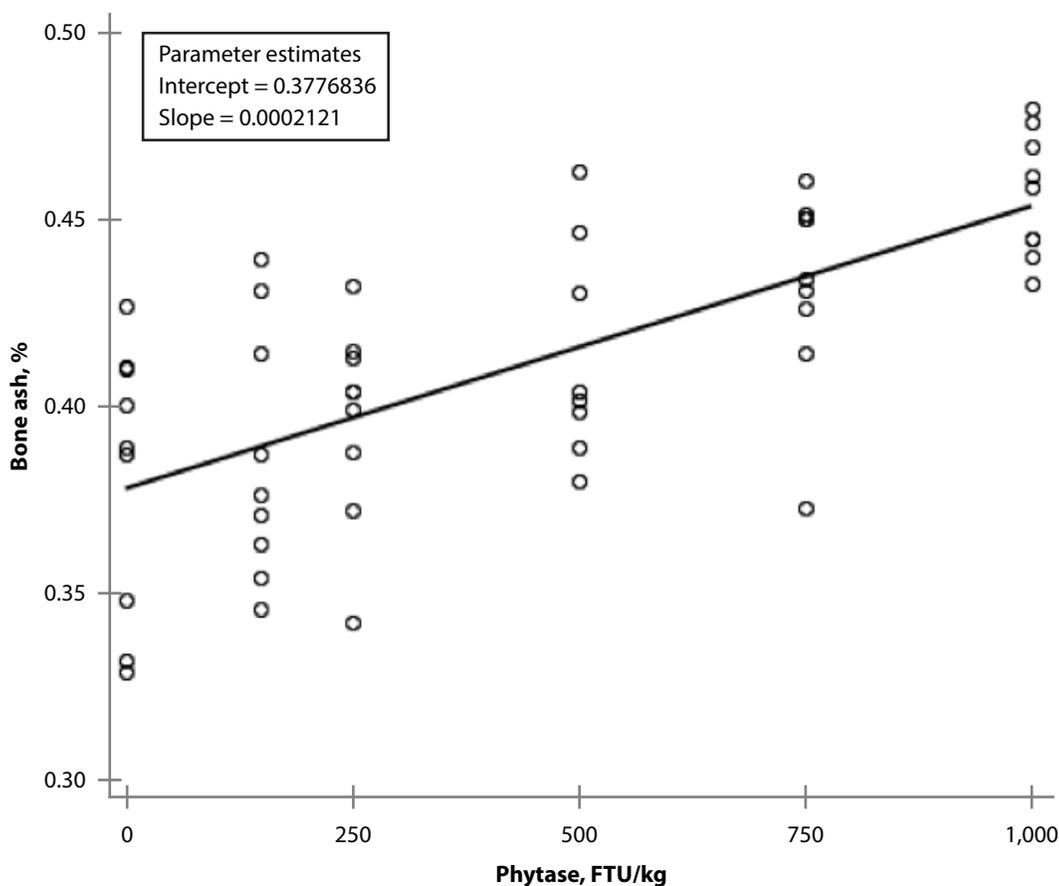
**Table 5. Calculated aP release values based on different response criteria**

Item	Phytase, FTU/kg <sup>1</sup>					SEM	Probability, <i>P</i> <	
	150	250	500	750	1000		Linear	Quadratic
ADG	0.036	0.042	0.050	0.079	0.103	0.009	0.001	0.325
F/G	0.025	0.046	0.072	0.064	0.109	0.014	0.001	0.226
Bone ash weight	-0.003	-0.036	0.042	0.073	0.159	0.008	0.001	0.206
Percent bone ash	0.000	0.034	0.093	0.144	0.227	0.032	0.001	0.737

<sup>1</sup>Natuphos E 5,000 G FTU/kg (BASF Corporation, Florham Park, NJ).

**Table 6. Available P release equations for Natuphos E 5,000 phytase based on various response criteria**

Response	aP release equation
Bone ash weight	aP release = 0.000116 × FTU/kg
Percentage bone ash	aP release = 0.000212 × FTU/kg



**Figure 1. Influence of Natuphos E 5,000 phytase level on percentage bone ash.**

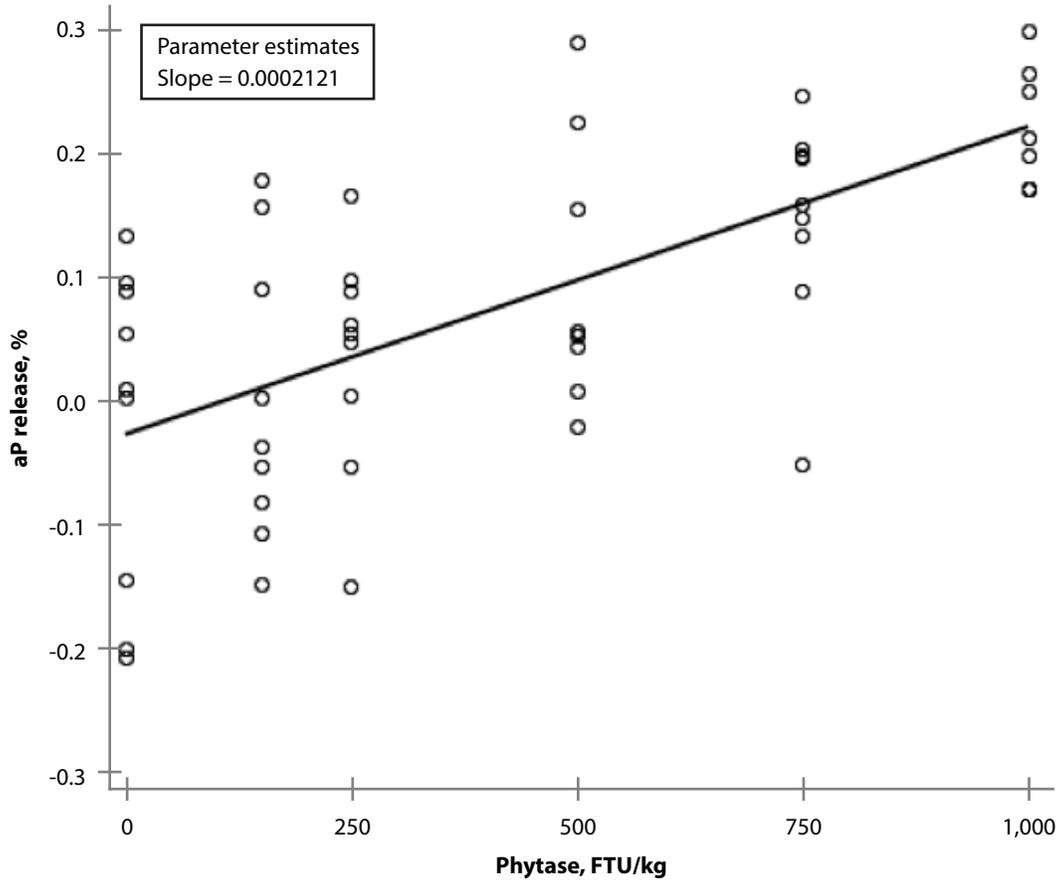


Figure 2. Influence of Natuphos E 5000 phytase level on available P (aP) release, calculated from percentage bone ash.

## Evaluating the Effect of Superdosing Natuphos E 5,000 G Phytase on Nursery Pig Performance<sup>1</sup>

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and R.D. Goodband*

### Summary

A total of 360 nursery pigs (DNA 200 × 400, initially 12.92 lb) were used in a 42-d growth trial to determine the effect of superdosing a novel phytase source (Natuphos E 5000 G, BASF Corporation, Florham Park, NJ). Pigs were randomly allotted to pens at weaning in a randomized complete block design to 1 of 8 dietary treatments. There were 5 pigs per pen and 9 pens per treatment. Diets were fed in 3 phases from d 0 to 7, 7 to 21, and 21 to 42. Dietary treatments were a negative control (NC) with 0.40, 0.30, or 0.25% aP from inorganic P for Phases 1, 2, and 3, respectively; and the NC with increasing phytase levels of 500, 1,000, 2,000, 3,000, or 4,000 phytase units (FTU)/kg. There was also a positive control (PC) with 0.55, 0.45, or 0.40% aP from inorganic P for Phases 1, 2, and 3, respectively, or the PC with 2,000 FTU/kg. On d 42, one pig per pen was euthanized and the right fibula was removed for bone ash analysis. From d 0 to 42, pigs fed increasing phytase in the negative control diet tended to have increased (quadratic,  $P = 0.064$ ) ADG resulting in heavier (linear,  $P = 0.082$ ) ending BW and improved (quadratic,  $P < 0.01$ ) F/G. Adding 2,000 FTU/kg phytase to the positive control diet did not influence ADG or ADFI, but tended to improve (linear,  $P = 0.068$ ) F/G. The NC diet with 500 FTU/kg and PC diets were formulated to be equivalent in available Ca and P. When comparing the two diets, pigs fed the positive control diet had increased (linear,  $P = 0.007$ ) ADFI; however, pigs fed the NC with 500 FTU/kg phytase diets had improved (linear,  $P = 0.034$ ) F/G. Bone ash weights were increased (quadratic,  $P < 0.001$ ) for pigs fed increasing phytase in the NC diets. Additionally, percentage bone ash values increased as phytase increased in the NC (linear,  $P < 0.001$ ) and PC ( $P < 0.001$ ) diets. There was a tendency for the PC diet to have greater ( $P = 0.099$ ) percentage bone ash when compared to the NC diet with 500 FTU/kg of phytase. In summary, this study shows that increasing dietary phytase increased percentage bone ash values, and a tendency for improved F/G as phytase was added to the positive control diet with P and Ca formulated at NRC (2012) recommendations. However, there was no further improvement in growth performance when phytase was included above 1,000 FTU/kg.

<sup>1</sup> Appreciation is expressed to BASF Corporation (Florham Park, NJ) for financial support of this experiment.

<sup>2</sup> Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.

Key words: nursery pig, phosphorus, phytase, superdose

## Introduction

Phosphorus is an important macro mineral in swine nutrition. Along with Ca and vitamin D, it contributes to bone development. Most swine diets are formulated with cereal grains, which contain P in the form of phytic acid. Monogastrics lack the enzyme necessary to cleave the phosphates from phytic acid for absorption. Thus, phytase enzyme is commonly added to swine diets to make P more available to the pig. This allows for reduced inclusion of P from inorganic P sources, like mono- and dicalcium phosphate, and reduced P excretion.

Superdosing phytase describes the concept of supplying phytase above a level needed to help meet the available P requirement. Previous studies have shown improved growth performance in nursery pigs fed superdose levels of phytase, with greater improvement seen when digestible P, amino acids and other nutrients were at marginal concentrations relative to the dietary predicted requirements.<sup>3</sup>

Natuphos E 5,000 G is a relatively new source of phytase available to the U.S. swine industry. In a previous nursery study, Natuphos E 5,000 G improved (linear,  $P < 0.01$ ) ADG, ADFI, F/G, and percentage bone ash as phytase increased from 0 to 1,000 FTU/kg. However, data are not available to determine the impact of feeding even higher levels of this new source of phytase. Therefore, the objective of this study was to evaluate the effect of superdosing Natuphos E 5000 G on the growth performance and percentage bone ash in nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the Kansas State University Segregated Early Wean Facility in Manhattan, KS. Two identical barns were environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

A total of 360 barrows (DNA 200 × 400, initially 12.92 lb) were used in a 42-d growth trial. Pigs were randomly allotted to pens and then pens of pigs were blocked by weight and randomly allotted to 1 of 8 dietary treatments. There were 5 pigs per pen and 9 replications (pens) per dietary treatment.

Dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Ingredients containing Ca and/or P were analyzed (Ca, P) prior to manufacturing diets in order to determine nutrient loading values (Table 1). Phytase premix was also analyzed to determine the inclusion rate in the experimental diets and contained 5,111,000 FTU of phytase/kg. Diets were fed in 3 phases from d 0 to 7, 7 to 21, and 21 to 42. Dietary treatments included a negative control with 0.40, 0.30, or 0.25% aP from inorganic P for Phases 1, 2, and

<sup>3</sup> Gonçalves MAD, Dritz SS, Tokach MD, et al. Fact sheets – comparing phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs. J. Swine Health Prod. 2016;24(2):97–101

3, respectively; the negative control plus increasing phytase levels of 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg in each phase; a positive control with 0.55, 0.45, or 0.40% aP from inorganic P for Phases 1, 2, and 3, respectively, or the positive control with 2,000 FTU/kg in each phase. The positive control was formulated with Ca and P to be similar to NRC (2012) recommendations for the weight range used. The negative control was formulated to be the positive control minus the Ca and P expected to be released by 500 FTU of Natuphos E phytase. The negative control with 500 FTU of phytase/kg and PC treatments were compared to determine the extra phosphoric effect of Natuphos E 5,000 G.

All dietary treatments were derived from 8 identical basal batches of ingredients by phase (Table 2). After manufacturing the basal batches, they were bagged off into 8 identical sets (200 lb of Phase 1, 800 lb of Phase 2, and 2,000 lb of Phase 3 per treatment). For each experimental diet, a subset of bags (50 lb each) from the basal diet were added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 3). During bagging of experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, and 35th bags, pooled, and used for phytase and nutrient analysis.

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for proximate nutrient analysis, including CP, Ca, and P (Table 4). In addition, one sample was sent to another commercial feed laboratory (Eurofins Scientific Inc., Des Moines, IA) for complete diet phytase analysis (AOAC; method 2000.12).

During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G. On d 42 of the study, the median weight pig in each pen was euthanized via captive bolt and fibulas were collected to determine bone ash value. Once collected, all fibulas were stored at  $-4^{\circ}\text{F}$ . To determine bone ash concentrations, bones were autoclaved for 60 min. Adhering tissue and cartilage caps were removed and bones were dried at  $221^{\circ}\text{F}$  for 7 d. Then dried fibulas were ashed in a muffle furnace at  $1,112^{\circ}\text{F}$  for 24 h to determine total ash weight and percentage bone ash.

### *Data Analysis*

All data (pen means or bone values) 3 SD outside the mean of each response criteria were considered outliers and were removed from analysis. In Phase 1, there were 4 pen outliers for F/G, 1 F/G outlier for Phase 2, and 1 F/G outlier for Phase 3. However, the pen data were retained for the evaluation of bone analysis data.

Data were analyzed as a randomized complete block design with pen as the experimental unit. Barn was treated as a random effect. Pre-planned contrast statements were utilized to determine the linear and quadratic responses of phytase. A pairwise comparison was used to compare the PC and PC + 2,000 FTU phytase treatments. Another pairwise comparison was used to compare the NC with 500 FTU of phytase/kg and the PC control. Analysis of variance was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Results were considered to be significant with  $P$ -values  $\leq 0.05$  and were considered to be a trend with  $P$ -values  $\leq 0.10$ .

## Results and Discussion

Chemical analysis of CP, Ca, and P of the experimental diets were similar to those expected from diet formulation. Analyzed phytase increased as phytase addition increased, but was greater than expected across all diets (Table 4).

From d 0 to 21, there were no differences observed for growth performance amongst dietary treatments. From d 21 to 42, adding phytase to the negative control diet tended to increase (quadratic,  $P = 0.078$ , Table 5) ADG and (linear,  $P = 0.095$ ) ADFI. In addition, F/G improved (quadratic,  $P = 0.007$ ) with increasing phytase in the NC diet. Among pigs fed the 2 positive control diets, including phytase at 2,000 FTU tended to improve ( $P = 0.056$ ) F/G. Pigs fed the PC had increased ( $P = 0.038$ ) ADG and ( $P = 0.049$ ) ADFI compared to those fed the NC. When comparing the NC diet with 500 FTU/kg phytase and the PC diet formulated to have the same aP, pigs fed the PC diet had increased ( $P < 0.05$ ) ADG and ADFI; however, pigs fed the NC with 500 FTU/kg of phytase had improved ( $P = 0.052$ ) F/G.

From d 0 to 42, pigs fed increasing phytase in the NC diet tended to have increased (quadratic,  $P = 0.064$ ) ADG resulting in heavier (linear,  $P = 0.082$ ) ending BW and improved (quadratic,  $P = 0.007$ ) F/G. Adding 2,000 FTU/kg phytase to the positive control diet did not influence ADG or ADFI, but tended to improve (linear,  $P = 0.068$ ) F/G. The NC diet with 500 FTU/kg and PC diets were formulated to be equivalent in available Ca and P. When comparing the two diets, pigs fed the positive control diet had increased (linear,  $P = 0.007$ ) ADFI; however, pigs fed the NC with 500 FTU/kg phytase diets had improved (linear,  $P = 0.034$ ) F/G.

Bone ash weights increased (quadratic,  $P < 0.001$ ) for pigs fed increasing phytase in the NC diets. In addition, percentage bone ash values increased as phytase increased in the NC (linear,  $P < 0.001$ ) and PC ( $P < 0.001$ ) diets. There was a tendency for pigs fed the PC diet to have greater ( $P = 0.099$ ) percentage bone ash when compared to the NC diet containing 500 FTU/kg of phytase. Overall, this study shows percentage bone ash values increased as added dietary phytase increased, and a tendency for improved F/G as phytase was added to the positive control diet when P and Ca were formulated at NRC (2012) recommendations. However, there was no improvement in growth performance when phytase was included above 1,000 FTU/kg in the negative control diet.

**Table 1. Analyzed ingredient composition<sup>1</sup> (as-fed basis)**

Ingredient <sup>2</sup>	Analyzed value, %	
	P	Ca
Corn	0.31	0.03
Soybean meal	0.72	0.43
Limestone	0.23	37.73
Monocalcium P	20.54	16.38
Fish meal	3.07	5.59
Dried whey	0.80	0.58
Blood plasma	1.00	0.19
HP 300 <sup>3</sup>	0.74	0.38
Corn DDGS, > 6 and < 9% oil	0.98	0.06
Trace mineral premix	0.03	18.28
Vitamin premix	0.04	18.17

<sup>1</sup>Duplicate ingredient samples were pooled and analysis was performed at a commercial laboratory (Ward Laboratory, Kearney, NE).

<sup>2</sup>Dairylac80 was not available for nutrient analysis prior to manufacturing. 0.27% Ca and 0.74% P were used for formulation.

<sup>3</sup>Hamlet Protein Inc. (Findlay, OH).

**Table 2. Composition of basal batch (as-fed basis)<sup>1</sup>**

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	36.80	52.09	62.98
Soybean meal	20.80	27.46	32.93
Dairylac 80 <sup>3</sup>	15.14	5.05	---
Dried whey	8.08	5.05	---
HP 300 <sup>4</sup>	5.05	5.05	---
Corn DDGS, > 6% and < 9% oil	5.05	---	---
Blood plasma	4.04	---	---
Fish meal	1.26	1.26	---
Choice white grease	1.01	1.01	1.01
Monocalcium P	0.28	0.56	0.86
Limestone	1.19	0.98	0.83
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.30	0.38	0.35
DL-Met	0.17	0.20	0.14
L-Thr	0.12	0.16	0.13
L-Val	---	0.05	
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Choline chloride 60%	0.04	---	---
Total	100.00	100.00	100.00
Calculated analysis			
SID <sup>2</sup> Lys, %	1.40	1.35	1.25
Total Lys, %	1.58	1.50	1.39
SID amino acid ratios			
Ile:Lys	58	60	61
Leu:Lys	122	118	125
Met:Lys	33	37	34
Met and Cys:Lys	57	58	56
Thr:Lys	63	63	62
Trp:Lys	19.3	17.8	18.0
Val:Lys	68	69	66
CP, %	22.7	22.2	21.8
NE, kcal/lb	1,141	1,123	1,105
SID Lys:ME, g/Mcal	4.12	4.03	3.79
Ca, %	0.71	0.66	0.56
P, %	0.66	0.62	0.60
Available P, %	0.40	0.30	0.25

<sup>1</sup>The basal batch was used as the major ingredient within each experimental diet.

<sup>2</sup>Standardized ileal digestible.

<sup>3</sup>International Ingredient Corporation (St. Louis, MO).

<sup>4</sup>Hamlet Protein Inc. (Findlay, OH).

**Table 3. Ingredient composition of experimental diets (as-fed basis)**

Ingredient, %	Experimental diet					
	Phase 1		Phase 2		Phase 3	
	Negative control	Positive control	Negative control	Positive control	Negative control	Positive control
Basal mix	96.52	96.52	98.43	98.43	98.75	98.75
Corn	3.35	2.52	1.46	0.63	1.10	0.25
Soybean meal	0.02	0.03	0.01	0.07	---	0.05
Limestone	---	0.73	---	0.08	---	0.08
Monocalcium P	---	0.05	---	0.70	---	0.75
Sand <sup>1</sup>	0.10	0.15	0.10	0.10	0.15	0.13
Phytase <sup>2</sup>	---	---	---	---	---	---
Calculated analysis						
CP, %	22.8	22.8	22.2	22.2	21.2	21.3
Ca, %	0.71	0.85	0.66	0.80	0.56	0.70
P, %	0.66	0.81	0.63	0.77	0.61	0.76
Ca:P ratio	1.07	1.05	1.05	1.04	0.93	0.92

<sup>1</sup>Sand was used to displace corn in the diet as experimental inclusion rate varied; as a result the same amount of basal mix was added to each of the treatment diets.

<sup>2</sup>Natuphos E 5,000 G FTU/kg (BASF Corporation, Florham Park, NJ) was added to the negative control to achieve experimental diets with 0, 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg or was added to positive control diets to achieve experimental diets with 0 or 2,000 FTU/kg. The phytase premix was analyzed for phytase level, and it contained 5,111,000 FTU/kg.

**Table 4. Analyzed composition of experimental diets (as-fed basis)<sup>1</sup>**

Diets	Analyzed composition			
	CP, %	Ca, %	P, %	Phytase, FTU/kg
Phase 1				
NC <sup>2</sup>	21.8	0.88	0.61	< 60
NC + 500 FTU	22.3	0.87	0.64	612
NC + 1000 FTU	22.1	0.89	0.63	1,100
NC + 2000 FTU	22.1	0.90	0.64	2,060
NC + 3000 FTU	22.4	0.93	0.64	3,880
NC + 4000 FTU	22.2	0.85	0.60	5,270
PC <sup>3</sup>	21.8	1.10	0.76	< 60
PC + 2000 FTU	22.4	1.07	0.80	2,580
Phase 2				
NC	21.8	0.75	0.59	< 60
NC + 500 FTU	21.6	0.78	0.58	650
NC + 1000 FTU	21.3	0.83	0.61	1,350
NC + 2000 FTU	21.9	0.84	0.63	2,590
NC + 3000 FTU	22.6	0.75	0.56	3,630
NC + 4000 FTU	22.6	0.89	0.67	5,200
PC	21.6	1.01	0.74	< 60
PC + 2000 FTU	22.2	0.94	0.75	2,560
Phase 3				
NC	20.8	0.75	0.63	< 60
NC + 500 FTU	22.0	0.75	0.61	536
NC + 1000 FTU	21.6	0.73	0.60	1,190
NC + 2000 FTU	21.5	0.78	0.61	2,280
NC + 3000 FTU	21.9	0.70	0.60	3,710
NC + 4000 FTU	21.8	0.70	0.63	4,660
PC	21.9	0.87	0.77	62
PC + 2000 FTU	22.2	0.87	0.77	2,110

<sup>1</sup>Dietary samples were pooled and proximate analysis was performed in triplicate by a commercial laboratory (Ward Laboratories, Kearney, NE). Additionally, phytase analysis was conducted to determine complete diet phytase concentrations at another commercial laboratory (Eurofins Scientific Inc., Des Moines, IA).

<sup>2</sup>Negative Control.

<sup>3</sup>Positive Control.

**Table 5. Effects of superdosing Natuphos E 5,000 G on nursery pig growth performance and bone ash values<sup>1</sup>**

Item	Negative control <sup>2</sup>						Positive control <sup>3</sup>		SEM	<i>P</i> <				
	0	500	1,000	2,000	3,000	4,000	0	2,000		Negative control		NC vs. PC	NC + 500 vs. PC <sup>4</sup>	PC vs. PC + 2000
										Linear	Quadratic			
BW, lb														
d 0	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	0.030	0.250	0.818	0.687	0.230	0.421
d 42	47.4	47.2	49.8	49.0	49.6	49.0	50.0	49.8	0.837	0.082	0.128	0.035	0.021	0.920
d 0 to 7														
ADG, lb	0.14	0.16	0.19	0.16	0.17	0.18	0.18	0.16	0.021	0.293	0.785	0.145	0.475	0.512
ADFI, lb	0.25	0.25	0.27	0.24	0.26	0.28	0.27	0.25	0.015	0.165	0.347	0.248	0.332	0.403
F/G	1.87	1.66	1.52	1.59	1.69	1.62	1.59	1.81	0.159	0.480	0.289	0.165	0.727	0.277
d 7 to 21														
ADG, lb	0.61	0.60	0.65	0.61	0.65	0.64	0.65	0.63	0.032	0.273	0.763	0.333	0.215	0.630
ADFI, lb	0.77	0.76	0.80	0.78	0.80	0.80	0.81	0.77	0.029	0.343	0.851	0.250	0.173	0.241
F/G	1.27	1.28	1.23	1.28	1.24	1.25	1.26	1.23	0.029	0.560	0.886	0.884	0.603	0.389
d 21 to 42														
ADG, lb	1.19	1.19	1.25	1.25	1.25	1.23	1.27	1.28	0.028	0.192	0.078	0.038	0.048	0.847
ADFI, lb	1.81	1.74	1.86	1.85	1.85	1.87	1.92	1.87	0.040	0.095	0.644	0.049	0.003	0.398
F/G	1.52	1.46	1.49	1.47	1.48	1.52	1.51	1.46	0.016	0.531	0.009	0.680	0.052	0.056
d 0 to 42														
ADG, lb	0.81	0.83	0.87	0.85	0.87	0.84	0.88	0.88	0.021	0.314	0.064	0.030	0.107	0.864
ADFI, lb	1.19	1.17	1.24	1.21	1.23	1.22	1.28	1.24	0.028	0.188	0.427	0.029	0.007	0.289
F/G	1.46	1.40	1.42	1.42	1.42	1.46	1.45	1.41	0.015	0.611	0.007	0.531	0.034	0.068
Bone ash, g <sup>5</sup>	1.94	2.30	2.35	2.56	2.53	2.25	2.42	2.51	0.093	0.012	0.001	0.001	0.372	0.465
Bone ash, %	44.2	45.2	47.1	48.0	48.4	49.1	47.0	51.3	0.007	0.001	0.078	0.010	0.099	0.001

<sup>1</sup>A total of 360 barrows (DNA 200 × 400, initially 12.92 lb) were used in a 42-d growth study with 5 pigs per pen and 9 pens per treatment (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ).

<sup>2</sup>Negative control diets were formulated with 0.40, 0.30, or 0.25% aP from inorganic P for Phases 1, 2, and 3, respectively. Phytase was added at 0, 500, 1,000, 2,000, 3,000, 4,000 FTU/kg to the negative control diet to achieve final experimental diets.

<sup>3</sup>Positive control diets were formulated with 0.55, 0.45, or 0.40% aP from inorganic P for Phases 1, 2, and 3, respectively. Phytase was added at either 0 or 2,000 FTU/kg to the positive control diet to achieve final experimental diets.

<sup>4</sup>NC diet was formulated to be the PC minus the Ca and P released by 500 FTU of Natuphos E phytase. The NC + 500 FTU and PC treatments were compared to determine the extra phosphoric effect of Natuphos E.

<sup>5</sup>One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

## Evaluation of Dietary Electrolyte Balance on Nursery Pig Performance<sup>1</sup>

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### Summary

A total of 2,880 pigs (PIC 327 × L42; initial BW 11.4 lb) were used in a 35-d growth performance trial evaluating the effects of dietary electrolyte balance (dEB) on growth performance of nursery pigs. There were 30 pigs per pen (60 pigs per double-sided feeder) and 12 replications (feeder) per treatment. Pens of pigs were allotted by BW and sex on arrival, and randomly assigned to 1 of 4 dietary treatments. Treatment diets were corn-soybean meal-based with dried whey and other specialty protein sources used in Phase 1 with decreased amounts in Phase 2. Dietary electrolyte balance was determined using the following equation:  $dEB = ((Na \times 434.98) + (K \times 255.74) - (Cl \times 282.06))$  mEq/kg. Phase 1 diets had dEB's of 84, 137, 190, and 243 mEq/kg. Phase 2 diets had dEB's of 29, 86, 143, and 199 mEq/kg. Limestone was used as the main Ca source in the high dEB diet and was replaced by increasing levels of CaCl<sub>2</sub> to form the other experimental diets. The lowest dEB diets were achieved by adding 1.17% and 1.25% CaCl<sub>2</sub> in Phase 1 and Phase 2, respectively. The highest dEB diets required additions of 0.55 and 0.80% limestone for Phases 1 and 2, respectively. The two intermediate diets were then balanced to have an equal stepwise increase in dEB. Dietary Ca concentrations were maintained in the three highest dEB diets, but increased in the low dEB diet with the increasing level of CaCl<sub>2</sub>. After d 21 of experimental diets, a common Phase 3 diet (Table 3) was fed to all pigs and was a typical nursery diet fed in commercial production with a dEB of 257 mEq/kg. From d 0 to 8 (Phase 1), decreasing dEB decreased (quadratic,  $P < 0.05$ ) ADG, ADFI, and final BW, and worsened (quadratic,  $P = 0.042$ ) F/G. Likewise, from d 8 to 21 (Phase 2), ADG (quadratic,  $P = 0.022$ ) and ADFI (linear,  $P = 0.011$ ) decreased as dEB was decreased, resulting in a worsening of feed efficiency (quadratic,  $P < 0.001$ ). From d 0 to 21, ADG and ADFI decreased (linear,  $P < 0.05$ ) as dEB decreased resulting in poorer (quadratic,  $P < 0.001$ ) F/G. When a common diet was fed from d 21 to 35 (Phase 3), pigs that were previously fed low dEB diets had improved (linear,  $P < 0.001$ ) ADG and F/G; however, no differences were observed for feed intake. Overall (d 0 to 35), decreasing dEB in nursery diets from d 0 to 21 caused a reduction in ADG and final BW (linear,  $P < 0.001$ ), which was the result of a tendency for lower ADFI (linear  $P = 0.077$ ) and poorer feed efficiency (quadratic,  $P = 0.028$ ).

<sup>1</sup> Appreciation is expressed to Jason Tebay and Dr. Matt Allerson, Holden Farms, for their technical support and expertise in conducting the experiment.

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In conclusion, feeding reducing levels of dietary dEB in nursery diets resulted in poorer growth performance of weanling pigs.

Key words: dietary electrolyte balance, growth performance, nursery pig

## Introduction

Electrolytes are key minerals that can be defined as chemical substances that separate when dissolved in fluids to form positive (cation) and negative (anion) ions. These charged ions produce an electrically conductive solution that serves as a medium for cellular signaling, biochemical reactions, transport of substrates across cellular membranes, and the removal of waste products from the body among others. Biologically, there are seven major electrolytes: (1)  $\text{Na}^+$ ; (2)  $\text{K}^+$ ; (3)  $\text{Mg}^{2+}$ ; (4)  $\text{Ca}^{2+}$ ; (5)  $\text{Cl}^-$ ; (6)  $\text{HPO}_4^{2-}$ ; (7)  $\text{HCO}_3^-$  (Alberts et al. 2002).<sup>3</sup>

Of the seven electrolytes, Mongin (1981)<sup>4</sup> determined that the sum of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  were the most influential minerals in contributing to the acid-base status of broilers. Based on these findings, an equation was derived in predicating the net acid intake of set animal referred to as dietary electrolyte balance ( $\text{dEB} = \text{Na} + \text{K} - \text{Cl}$  mEq/kg). Traditionally, the optimal electrolyte balance in the diet for pigs is reported to be around 250 mEq/kg (NRC, 2012)<sup>5</sup> but limited research exists in this particular area. Recently, Guzmán-Pino et al. (2015),<sup>6</sup> reported that nursery pigs fed diets with increasing dEB had poorer ADG and F/G when dEB exceeded 150 mEq/kg. These researchers used  $\text{CaCl}_2$  to lower the dEB and reported 48.7% improvement in ADG by decreasing dEB from 269 to 16 mEq/kg. Thus, the objective of this study was to determine the influence of dEB on growth performance in nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The experiment was conducted at a commercial nursery in MN. Pigs were housed in pens (6 × 11 ft) that were equipped with double sided, 5-hole, stainless steel dry feeder and one cup waterer for ad libitum access to feed and water. Feed additions were made by a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that measured feed amounts for each feeder.

A total of 2,880 pigs (PIC 327 × L42; initial BW 11.4 lb) with 30 pigs per pen (60 pigs per feeder) and 12 replications (feeder) per treatment were used in a 35-d trial evaluating the effects of dEB on growth performance of nursery pigs. Pens of pigs were blocked by BW and gender to 1 of 4 treatments in a completely randomized block design. Pigs and feeders were weighed on d 0, 8, 15, 21, and 35 of the trial to determine ADG, ADFI, and F/G.

<sup>3</sup> Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2002. *Molecular biology of the cell*. 4<sup>th</sup> edition, Garland Science, New York.

<sup>4</sup> Mongin, P. 1981. Recent advances in dietary anion-cation balance: Applications in poultry. *Proc. Nutr. Soc.* 40:285-294.

<sup>5</sup> *Nutrient requirements of swine*. 2012. 11th edition, National Research Council, Washington DC.

<sup>6</sup> Guzmán, S. A., D. Solà-Oriol, R. Daving, E. G. Manzanilla, and J. F. Pérez. 2015. Influence of dietary electrolyte balance on feed preference and growth performance of post-weaned piglets. *J. Anim. Sci.* 93:2840-2848. doi:10.2527/jas2014-8380

Experimental diets (Tables 1 and 2) were fed in two phases, with the first phase being provided at 3 lb/pig at weaning. The second phase was fed until d 21 post-weaning, when pigs weighed approximately 22 lb. Treatment diets were corn-soybean meal-based with dried whey and other specialty protein sources used in Phase 1 and with decreased amounts in Phase 2.

Dietary electrolyte balance was determined using the following equation:  $((\text{Na} \times 434.98) + (\text{K} \times 255.74) - (\text{Cl} \times 282.06))$ .<sup>4</sup> Phase 1 diets had dEB's of 84, 137, 190, and 243 mEq/kg. Phase 2 diets had dEB's of 29, 86, 143, and 199 mEq/kg. Limestone was used as the main Ca source in the high dEB diet and was replaced by increasing levels of CaCl<sub>2</sub> to form the other experimental diets. The lowest dEB diets were achieved by adding 1.17% and 1.25% CaCl<sub>2</sub> in Phase 1 and Phase 2, respectively. The highest dEB diets required additions of 0.55 and 0.80% limestone for Phases 1 and 2, respectively. The two intermediate diets were then balanced to have an equal stepwise increase in dEB. Dietary Ca concentrations were maintained in the three highest dEB diets, but increased in the low dEB diet with the increasing level of CaCl<sub>2</sub>. After d 21, a common Phase 3 diet (Table 3) was fed to all pigs and was typical of a standard nursery diet fed in commercial production with a dEB of 257 mEq/kg. Phase 1 diets were fed in pellet form, while Phases 2 and 3 were fed in meal form.

Diet samples were taken from 6 feeders per dietary treatment on each weigh day and combined to form a composite sample within each phase. Samples were then stored at -4°F until further analysis. Samples of the diets were analyzed for DM, CP, crude fat, Na, K, Cl, Ca and P (Ward Laboratory, Kearney, NE). All samples for all assays were analyzed in duplicate and were within 10% error of each other. Following chemical analysis, analyzed values for Na, K, and Cl were used to calculate dEB.

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with feeder as the experimental unit. Dietary treatments were the fixed effect and block and room served as the random effect in the model. The effects of increasing dietary dEB on performance criteria were determined by linear and quadratic polynomial contrasts. A  $P$ -value  $\leq 0.05$  was considered significant and  $0.05 < P \leq 0.10$  was considered a tendency.

## Results and Discussion

Chemical analysis of experimental diets showed that most nutrients were similar to formulated values for Phase 1 diets (Table 4). Analyzed values for Na and K in Phase 2 diets were consistently higher across dietary treatments; however, Cl levels were lower than formulated values. Although these values varied from formulated values, the range of dEB targeted was ultimately maintained across dietary treatments.

From d 0 to 8 (Phase 1), decreasing dEB decreased (quadratic,  $P < 0.05$ ) ADG, ADFI, and final BW, and worsened (quadratic,  $P = 0.042$ ) F/G. Likewise, from d 8 to 21 (Phase 2), ADG (quadratic,  $P = 0.022$ ) and ADFI (linear,  $P = 0.011$ ) decreased as dEB was decreased, resulting in a worsening of feed efficiency (quadratic,  $P < 0.001$ ).

From d 0 to 21, ADG and ADFI decreased (linear,  $P < 0.05$ ) as dEB decreased resulting in poorer (quadratic,  $P < 0.001$ ) F/G. When a common diet was fed from d 21 to 35

(Phase 3), pigs that were previously fed low dEB diets had improved (linear,  $P < 0.001$ ) ADG and F/G; however, no differences were observed for feed intake.

Overall (d 0 to 35), decreasing dEB in nursery diets from d 0 to 21 caused a reduction in ADG and final BW (linear,  $P < 0.001$ ), which was the result of a tendency for lower ADFI (linear  $P = 0.077$ ) and poorer feed efficiency (quadratic,  $P = 0.028$ ).

In conclusion, feeding diets with reduced dEB in nursery diets resulted in poorer growth performance of weanling pigs. A possible explanation for the poorer growth performance observed in the pigs fed low dEB diets could be attributed to the  $\text{CaCl}_2$  used to lower dEB. Sensory tests using humans has demonstrated that calcium chloride itself is perceived as bitter and metallic in taste (Lawless et al., 2003; 2004).<sup>7,8</sup> Yen et al. (1981)<sup>9</sup> found that high dietary  $\text{CaCl}_2$  limited intake in pigs through a Cl-induced metabolic acidosis. In addition, a preference trial conducted by Guzmán-Pino et al. (2015)<sup>6</sup> examining a low (with  $\text{CaCl}_2$ ) and high (without  $\text{CaCl}_2$ ) dEB diet showed that pigs preferred the high dEB diet. However, when those diets were used in a growth performance trial, performance decreased when pigs were fed levels greater than 150 mEq/kg of the diet. Personal contact with Guzmán-Pino and Davin indicated that a similar unprotected  $\text{CaCl}_2$  source was used in both experiments as well as the same equation in calculating dEB. The reasons for the observed improvement in growth rate and feed intake in their growth performance trial when pigs were fed low dEB diets are unclear. Therefore, further research is needed to understand the reason that Guzmán-Pino et al. (2015)<sup>6</sup> reported improved performance when  $\text{CaCl}_2$  was used to lower the dEB of diets, whereas the results of our experiment found the opposite effect.

<sup>7</sup> Lawless, H. T., F. Rapacki, J. Horne, and A. Hayes. 2003. The taste of calcium and magnesium salts and anionic modifications. *Food Qual. Prefer.* 14:319-325. doi:10.1016/S0950-3293(02)00128-3.

<sup>8</sup> Lawless, H. T., F. Rapacki, J. Horne, A. Hayes, and G. Wang. 2004. The taste of calcium chloride in mixtures with NaCl, sucrose, and citric acid. *Food Qual. Prefer.* 15:83-89. doi:10.1016/S0950-3293(03)00099-5.

<sup>9</sup> Yen, J. T., W. G. Pond, and R. L. Prior. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. *J. Anim. Sci.* 4:778-782. doi:10.2134/jas1981.524778

**Table 1. Phase 1 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Dietary electrolyte balance (mEq/kg) <sup>2</sup>			
	84	137	190	243
Corn	38.58	39.00	39.14	39.24
Soybean meal, 46.5% CP	17.71	17.68	17.67	17.66
Corn DDGS <sup>3</sup>	5.00	5.00	5.00	5.00
Fish meal	4.50	4.50	4.50	4.50
HP 300 <sup>4</sup>	2.50	2.50	2.50	2.50
Spray dried whey	25.00	25.00	25.00	25.00
Choice white grease	3.00	3.00	3.00	3.00
Monocalcium P, 21% P	0.40	0.40	0.40	0.40
Limestone	---	---	0.26	0.55
Calcium chloride	1.17	0.78	0.39	---
Sodium chloride	0.30	0.30	0.30	0.30
L-Lys HCL	0.48	0.48	0.48	0.48
MHA <sup>5</sup>	0.29	0.29	0.29	0.29
L-Thr	0.20	0.20	0.20	0.20
L-Trp	0.05	0.05	0.05	0.05
L-Val	0.10	0.10	0.10	0.10
Choline chloride, 60%	0.04	0.04	0.04	0.04
Phytase <sup>6</sup>	0.04	0.04	0.04	0.04
Zinc oxide	0.39	0.39	0.39	0.39
Selenium, 0.06%	0.05	0.05	0.05	0.05
Trace mineral premix	0.13	0.13	0.13	0.13
Vitamin premix	0.10	0.10	0.10	0.10
Total	100	100	100	100

*continued*

**Table 1. Phase 1 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Dietary electrolyte balance (mEq/kg) <sup>2</sup>			
	84	137	190	243
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.40	1.40	1.40	1.40
Ile:Lys	55	55	55	55
Leu:Lys	111	111	111	111
Met:Lys	40	40	40	40
Met and Cys:Lys	59	59	59	59
Thr:Lys	64	64	64	64
Trp:Lys	19	19	19	19
Val:Lys	67	67	67	67
ME, kcal/lb	1,570	1,576	1,578	1,580
CP, %	20.99	21.01	21.01	21.02
Na, %	0.39	0.39	0.39	0.39
Cl, %	1.34	1.16	0.97	0.78
K, %	1.14	1.14	1.14	1.14
Ca, %	0.84	0.73	0.73	0.73
P, %	0.67	0.67	0.67	0.67
Available P, %	0.59	0.59	0.59	0.59

<sup>1</sup>Phase 1 diets were fed for 8 d or to approximately 12.5 lb BW.

<sup>2</sup>Dietary electrolyte balance was calculated using the following equation:  $((\text{Na} \times 434.98) + (\text{K} \times 255.74) - (\text{Cl} \times 282.06))$ .

<sup>3</sup>Dried distillers grains with solubles,

<sup>4</sup>Hamlet Protein (Findlay, OH).

<sup>5</sup>Novus International (Saint Charles, MO).

<sup>6</sup>Quantum Blue (AB-Vista Americas, Plantation, FL).

**Table 2. Phase 2 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Dietary cation anion difference (mEq/kg) <sup>2</sup>			
	29	86	142	199
Corn	46.92	47.16	47.28	47.41
Soybean meal, 46.5% CP	24.70	24.68	24.67	24.66
Corn DDGS <sup>3</sup>	15.00	15.00	15.00	15.00
LOL Strbase LA S01J <sup>4</sup>	5.00	5.00	5.00	5.00
Fish meal	3.75	3.75	3.75	3.75
Choice white grease	1.00	1.00	1.00	1.00
Dicalcium P, 18.5% P	0.63	0.63	0.63	0.63
Limestone	---	0.20	0.50	0.80
Calcium chloride	1.25	0.83	0.42	---
Sodium chloride	0.35	0.35	0.35	0.35
L-Lys HCL	0.40	0.40	0.40	0.40
L-Thr	0.13	0.13	0.13	0.13
L-Trp	0.03	0.03	0.03	0.03
Zinc oxide	0.25	0.25	0.25	0.25
Iron oxide	0.10	0.10	0.10	0.10
CTC-100 <sup>5</sup>	0.20	0.20	0.20	0.20
Vitamin and TM premix	0.30	0.30	0.30	0.30
Total	100	100	100	100

*continued*

**Table 2. Phase 2 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Dietary cation anion difference (mEq/kg) <sup>2</sup>			
	29	86	142	199
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.35	1.35	1.35	1.35
Ile:Lys	61	61	61	61
Leu:Lys	129	129	129	129
Met:Lys	31	31	31	31
Met and Cys:Lys	57	57	57	57
Thr:Lys	63	63	63	63
Trp:Lys	19	19	19	19
Val:Lys	69	69	69	69
ME, kcal/lb	1,420	1,424	1,425	1,427
CP, %	23.5	23.5	23.6	23.6
Na, %	0.22	0.22	0.22	0.22
Cl, %	0.99	0.79	0.59	0.39
K, %	0.84	0.84	0.84	0.84
Ca, %	0.83	0.79	0.79	0.79
P, %	0.65	0.65	0.65	0.65
Available P, %	0.36	0.36	0.36	0.36

<sup>1</sup>Phase 2 diets were fed from approximately 12.5 lb to approximately 22 lb BW.

<sup>2</sup>Dietary electrolyte balance was calculated using the following equation:  $((\text{Na} \times 434.98) + (\text{K} \times 255.74) - (\text{Cl} \times 282.06))$ .

<sup>3</sup>Dried distillers grains with solubles.

<sup>4</sup>Land O' Lakes, Inc. (Purina Mills, LLC, Shoreview, MN).

<sup>5</sup>Zoetis Animal Health (Florham Park, NJ).

**Table 3. Phase 3 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	
Corn	38.33
Soybean meal, 46.5% CP	31.99
DDGS <sup>2</sup>	25.00
Choice white grease	1.00
Limestone	1.25
Dicalcium P, 18.5% P	1.03
Salt	0.50
L-Lys HCl	0.40
DL-Met	0.11
L-Thr	0.10
Vitamin and TM premix	0.30
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lys	1.35
Met:Lys	35
Met and Cys:Lys	59
Thr:Lys	64
Trp:Lys	18
Val:Lys	74
ME, kcal/lb	1,487
CP, %	25.39
Na, %	0.29
Cl, %	0.47
K, %	1.03
Ca, %	0.83
P, %	0.66
dEB, mEq/kg <sup>3</sup>	257
Available P, %	0.37
Analyzed composition, %	
DM	88.37
CP	22.48
Crude fat	5.90
Ca	0.82
P	0.64

<sup>1</sup>Phase 3 diets were fed from d 21 to 35 post-weaning.

<sup>2</sup>Dried distillers grain with solubles.

<sup>3</sup>Dietary electrolyte balance was calculated using the following equation:  $((\text{Na} \times 434.98) + (\text{K} \times 255.74) - (\text{Cl} \times 282.06))$ .

**Table 4. Chemical analysis of Phase 1 experimental diets<sup>1</sup>**

Item, %	dEB, mEq/kg			
	84	137	190	243
DM	90.54	90.73	91.22	90.81
CP	20.95	20.85	21.10	20.95
Crude fat	4.60	4.80	4.70	4.70
Na	0.36	0.43	0.45	0.39
K	1.26	1.26	1.28	1.25
Cl	1.36	1.21	0.99	0.80
Ca	1.02	0.98	0.95	0.90
P	0.75	0.67	0.72	0.72
dEB, mEq/kg <sup>2</sup>	95	168	244	264

<sup>1</sup>Complete diet samples were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, crude fat, Na, Cl, K, Ca, and P.

<sup>2</sup>Dietary electrolyte balance was calculated using the following formula: ((Na\*434.98) + (K\*255.74) – (Cl\*282.06)).

**Table 5. Chemical analysis of Phase 2 experimental diets<sup>1</sup>**

Item, %	dEB, mEq/kg <sup>2</sup>			
	29	86	142	199
DM	88.16	88.71	88.71	88.36
CP	21.00	23.15	23.55	21.35
Crude fat	5.20	5.10	5.30	5.20
Na	0.33	0.35	0.30	0.30
K	0.93	0.94	1.06	1.00
Cl	1.11	1.13	0.85	0.77
Ca	1.33	1.57	1.40	1.59
P	0.68	0.86	0.87	0.82
dEB, mEq/kg <sup>2</sup>	68	74	162	169

<sup>1</sup>Complete diet samples were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, crude fat, Na, Cl, K, Ca, and P.

<sup>2</sup>Dietary electrolyte balance was calculated using the following equation: ((Na\*434.98) + (K\*255.74) – (Cl\*282.06)).

**Table 6. Evaluation of dietary electrolyte balance on nursery pig performance<sup>1</sup>**

	mEq/kg				SEM	Probability, <i>P</i> <		
	Phase 1:	84	137	190		243	Linear	Quadratic
	Phase 2:	29	86	142	199			
BW, lb								
d 0		11.4	11.4	11.5	11.4	0.12	0.773	0.448
d 8		12.4	12.3	12.6	12.8	0.10	0.001	0.035
d 21		20.7	21.6	22.1	22.5	0.19	0.001	0.177
d 35		34.3	34.7	35.1	35.3	0.26	0.001	0.552
d 0 to 8								
ADG, lb		0.12	0.11	0.13	0.16	0.011	0.001	0.001
ADFI, lb		0.19	0.18	0.18	0.21	0.008	0.008	0.004
F/G		1.70	1.93	1.50	1.32	0.106	0.001	0.042
d 8 to 21								
ADG, lb		0.62	0.69	0.71	0.74	0.012	0.001	0.022
ADFI, lb		0.79	0.80	0.80	0.83	0.013	0.011	0.469
F/G		1.27	1.16	1.13	1.13	0.013	0.001	0.001
d 0 to 21								
ADG, lb		0.43	0.47	0.48	0.52	0.007	0.001	0.807
ADFI, lb		0.55	0.56	0.56	0.59	0.008	0.003	0.103
F/G		1.30	1.21	1.15	1.14	0.013	0.001	0.001
d 21 to 35								
ADG, lb		0.97	0.94	0.93	0.91	0.010	0.001	0.376
ADFI, lb		1.32	1.32	1.33	1.31	0.016	0.891	0.461
F/G		1.36	1.41	1.43	1.43	0.014	0.001	0.124
d 0 to 35								
ADG, lb		0.64	0.65	0.66	0.68	0.007	0.001	0.736
ADFI, lb		0.86	0.86	0.86	0.88	0.010	0.077	0.594
F/G		1.32	1.28	1.26	1.25	0.009	0.001	0.028

<sup>1</sup>A total of 2,880 pigs (PIC 327 × 1050; initial BW 11.4 lb) with 30 pigs per pen (60 pigs per feeder) and 12 replications per treatment were used in a 35-d growth performance trial. All experimental diets were fed in two phases (d 0 to 8, and d 8 to 21) with a common diet fed from d 21 to 35.

## Effects of Feeding a Finishing Diet Blended with Different Phases of Nursery Diets on Growth Performance and Economics of Nursery Pigs<sup>1</sup>

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### Summary

A total of 1,260 weaned pigs (PIC TR4 × (Fast LW × PIC L02); initially 12.9 lb BW) were housed in a commercial research barn and used in a 47-d study to determine the effects of blending a finishing diet into different phases of nursery diets on pig growth performance. Pens of pigs were blocked by initial BW and gender and allotted to 1 of 4 treatment groups (15 pens/treatment). In a 5-phase feeding program, the 4 treatments were: 1) standard nursery diets throughout (control); or standard nursery diets with 5.5 lb/pig of late finishing feed blended at the beginning of 2) Phase 2; 3) Phase 3; or 4) Phase 4. Phase changes were based on feed budgets. From d 0 to 7, all pigs received the same standard Phase 1 diet and had similar growth performance. Compared with pigs from control, blending finishing feed into the Phase 2 period resulted in poorer ( $P < 0.01$ ) ADG, ADFI, and F/G from d 7 to 14, poorer ( $P = 0.025$ ) F/G from d 21 to 28, decreased ( $P = 0.028$ ) ADG from d 28 to 35, and decreased ( $P < 0.05$ ) ADFI and F/G from d 35 to 47. Blending finishing feed during Phase 3 resulted in worsened ( $P < 0.001$ ) ADG and F/G from d 14 to 21, decreased ( $P = 0.010$ ) ADG from d 21 to 28, and lower ( $P < 0.05$ ) ADFI and F/G from d 35 to 47 compared with control pigs. Pigs that received blended diet in Phase 4 had impaired ( $P < 0.001$ ) ADG and F/G from d 21 to 28, but had improved ( $P = 0.010$ ) F/G from d 35 to 47. Overall (d 0 to 47), blending the finishing diet into Phase 2 decreased ( $P < 0.05$ ) ADG, ADFI, and final BW, but did not affect F/G compared with control pigs or pigs that had finishing feed blended into the Phase 4. Blending finishing feed into Phase 3 or 4 did not influence overall growth performance. Pigs that had finishing feed blended into Phase 2 or 3 had lower ( $P < 0.05$ ) overall feed costs than pigs from control and Phase 4 blending treatments. Gain value was decreased ( $P < 0.05$ ) when finishing feed was blended into Phase 2 compared with the control or when feed was blending into Phase 4. However, no differences in feed cost per lb of gain and only numerical differences in income over

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feed cost were observed among the treatments. In conclusion, feeding finishing feed in early nursery phase negatively affected pig growth performance; however, blending approximately 5.5 lb/pig finishing feed into nursery diets for pigs greater than 22 lb BW did not affect overall growth performance.

Key words: blending, finishing feed, nursery feed, growth, nursery pig

## Introduction

In a wean-to-finish pig production, one of the challenges in feed management is determining what to do with feed remaining in the bin at the end of the finishing phase. The precision of budgeting finishing feed based on predicted feed intake and closeout dates is not perfect. Thus, there is often feed remaining in the bins that must be removed and transported to another site or fed to the next group of pigs. However, in a wean-to-finish barn this happens to be newly weaned nursery pigs. A common strategy is to blend leftover finishing feed into late nursery diets, which requires prolonged feed storage and may result in tandem blending of the early nursery phase diets. Therefore, information on the timing of blending finishing feed into nursery diets is needed to quantify and mitigate the negative impact. This study was designed to replicate a common field scenario where 6 tons of the last finishing diet was left in the bins at a 2,200-head barn. Thus, approximately 5.5 lb finishing feed would be fed to each nursery pig in the subsequent turn. The objective of this study was to determine the effects of feeding finishing feed blended into different phases of nursery feed on nursery pig growth performance and production economics.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in the experiment. The study was conducted at New Fashion Pork's nursery research facility located in southwest Minnesota. The barn was equipped with pens ( $8.5 \times 18.25 \text{ ft}^2$ ) that contained a 3-hole dry self-feeder and a cup waterer to allow for ad libitum access to feed and water. Diets were manufactured at the New Fashion Pork feedmill located in Worthington, MN.

A total of 1,260 weaned pigs (PIC TR4  $\times$  (Fast LW  $\times$  PIC L02); initially 12.9 lb BW) were used. Pens of pigs (21 pigs/pen, 30 pens of barrows, and 30 pens of gilts) were blocked by initial BW and gender. Within blocks, pens were allotted randomly to 1 of 4 treatments with 15 replications per treatment. Pigs were fed a 5-phase feeding program (Table 1) with phase changes made by using feed budgets (Table 2). Treatments consisted of a standard 5-phase nursery diet program (control) and the standard program with 5.5 lb of the last finishing diet blended at the beginning of Phase 2, 3, or 4. In the blended diets, feed delivery followed the sequence of 2.75 lb/pig of late finishing feed, a 50:50 blend of late finishing and standard diet, and ended with the remaining budget of the standard nursery diet.

Feed additions to each individual pen were delivered and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN). Pens were weighed and feed disappearance was measured every 7 d to determine ADG, ADFI, and F/G. Nine feed samples (5 standard nursery diets, 1 finishing diet, and 3 blended diets) were collected

directly from the feed robot delivery outlet. Feed samples were delivered to the Kansas State University Swine Laboratory, stored at  $-68^{\circ}\text{F}$ , and analyzed for DM, CP, and mineral contents (Ward Laboratories, Inc., Kearney, NE).

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The statistical model included the fixed effect of blending phase and random effects of weight block and gender. Calculation of economics were based on a gain value of  $\$0.60/\text{lb}$  and feed prices of  $\$521$ ,  $\$449$ ,  $\$389$ ,  $\$297$ ,  $\$265$ , and  $\$172/\text{ton}$  of nursery Phase 1, 2, 3, 4, 5, and late finishing diets respectively. Results were considered significant at  $P < 0.05$  and marginally significant at  $0.05 < P < 0.10$ .

## Results and Discussion

As expected, the finishing diet contained lower CP, Ca, and P concentrations than nursery diets (Table 3). Nutrient concentrations in blended diets approximated to the average between the finishing diet and the corresponding standard nursery diet phase, indicating that diets were properly blended.

From d 0 to 7 (all received standard Phase 1 diet), there were no differences in growth performance and d 7 BW as expected (Table 4). From d 7 to 14 (Phase 2 diets), pigs that received late finishing feed blended into the Phase 2 diet had poorer ( $P < 0.01$ ) ADG, ADFI, F/G, and d 14 BW compared with pigs in other treatment groups. From d 14 to 21 (Phase 3 budgets), blending late finishing feed into the Phase 3 diet resulted in poorer ( $P < 0.01$ ) ADG and F/G than other treatments, but no differences in ADFI were observed. Body weights of pigs fed late finishing diet blended into Phase 2 or Phase 3 were lower ( $P < 0.05$ ) than pigs from control and Phase 4 blending treatments on d 21.

Between d 21 and 28 the switch from the Phase 3 to Phase 4 budgets occurred in the majority of the pens. During this period, ADG of pigs with late finishing feed blended into the Phase 3 or Phase 4 diets was lower ( $P < 0.05$ ) than that of pigs from control, but was not different from pigs in Phase 2 blending treatment. No difference in ADG among pigs from control and Phase 2 blending treatment was observed. Pigs with late finishing feed blended into the Phase 3 diet had lower ( $P = 0.002$ ) ADFI than pigs from the Phase 4 blending treatment with pigs from the control and Phase 2 blending treatments being intermediate. Pigs receiving late finishing feed blended into the Phase 4 blended diet had poorer ( $P < 0.01$ ) F/G than pigs from other treatments. Also, F/G of pigs from Phase 2 blending treatment was poorer ( $P = 0.025$ ) than that of pigs from the control, but was not different from pigs from the Phase 3 blending treatment. On d 28, BW of pigs that received late finishing feed blended into the Phase 2 or Phase 3 diets was lower ( $P < 0.05$ ) than pigs from control and Phase 4 blending treatments.

From d 28 to 35, the majority of the pens were fed their Phase 4 budgets with the diet change from Phase 4 to 5 occurring at the end of this period. A tendency for a treatment effect was observed for ADG with pigs that had received finishing feed blended into the Phase 2 diet having decreased ( $P < 0.05$ ) ADG compared with pigs from other treatment groups; however, no differences in ADFI and F/G were observed. On d 35, BW of pigs that received late finishing feed blended during Phase 2 was lower

( $P < 0.01$ ) than pigs from control and Phase 4 blending treatments, but was not different from pigs from Phase 3 blending treatment. Pigs that received late finishing feed blended into the Phase 3 diet also had lower ( $P = 0.013$ ) BW than pigs from the control treatment. Pigs receiving late finishing feed blended into the Phase 4 diet had similar BW compared with control pigs on d 35.

From d 35 to 47, all pigs were fed a standard Phase 5 diet. Average daily gain was similar among treatments. Pigs receiving late finishing feed blended into the Phase 2 or Phase 3 diets had decreased ( $P < 0.05$ ) ADFI compared with control pigs, but they were not different from pigs from Phase 4 blending treatment. Feed efficiency was improved ( $P < 0.01$ ) in pigs that previously had late finishing feed blended into their diets compared with the control. Pigs from Phase 3 blending treatment also had better ( $P = 0.020$ ) F/G than pigs from Phase 4 blending treatment.

Overall, blending finishing diet during Phase 2 resulted in decreased ( $P < 0.05$ ) ADG, ADFI, and final BW, but did not affect F/G compared with control pigs or pigs that had late finishing diet blended diet into the nursery Phase 4. No differences in growth performance were observed among pigs from control, Phase 3 blending, and Phase 4 blending treatments.

Blending the finishing diet in Phase 2 decreased growth performance immediately and the negative effects persisted during the subsequent periods. Pigs in early nursery phases are in an energy deficient state and their growth performance is highly dependent on the feed intake. Late finishing diets contain less special protein ingredients and is less palatable, which may be responsible to a low ADFI of young pigs. In addition, late finishing diets are low in AA, Ca, and P concentrations that are below the requirements of nursery pigs and prevent pigs from achieving maximum growth performance. When finishing feed was blended in Phase 3 or Phase 4, decreased growth performance was also observed. However, pigs receiving the blended diets in the later phases were able to maintain or increase feed intake to compensate partly for the negative impact of consuming the late finishing diet. Therefore, these pigs resumed the growth performance to the control level faster and in a greater degree compared with pigs receiving the finishing diet during Phase 2. Interestingly, pigs that received blended diets expressed superior feed efficiency compared with pigs fed no blended diets from d 35 to 47, which might be a result of the decreased feed intake and compensatory gain, but further investigation is needed to fully explain this observation.

Economic analysis is presented in Table 5. Blending finishing feed into Phase 2 or 3 decreased ( $P < 0.05$ ) feed cost relative to control pigs and pigs that received blended diet in Phase 4 which can be explained by the slightly decreased overall feed intake and lower cost of the late finishing diet. The lower final BW also caused pigs that received late finishing diet during Phase 2 to have lower ( $P < 0.05$ ) gain value than pigs from control and Phase 4 blending treatments, with no differences in gain value observed among control, Phase 3 blending, and Phase 4 blending treatments. No treatment effect was observed for feed cost per lb of gain. Income over feed cost was numerically decreased in pigs fed blended diets, and the magnitude was greater when pigs received the blended diet at a younger age; however, no significant difference was detected. Based on standard labor and transportation costs, approximately \$500 is need to reclaim 6 tons

of finishing feed to a feed mill located 40 miles away from the barn. In this scenario, the reclaim cost per pig (\$0.23) is less than the numerical reductions in income over feed cost when blending finishing feed into Phase 2 (\$0.69/pig), Phase 3 (\$0.42/pig), and Phase 4 (\$0.32/pig).

In summary, growth performance of nursery pigs was promptly influenced when blended finishing and nursery diets were fed, and its magnitude depended on which phase the finishing feed was blended in. However, for pigs greater than 22 lb BW, blending approximately 5.5 lb/pig finishing feed into nursery diets did not affect overall growth performance. Based on numerical differences observed in income over feed cost, it was not economical to feed 5.5 lb/pig of leftover finishing feed to nursery pigs in the test scenario.

**Table 1. Composition of experimental diets (as-fed basis)**

Items	Phase 2	Phase 3	Phase 4	Phase 5	Finishing
Ingredients, %					
Corn	43.14	39.27	37.07	38.39	79.00
Soybean meal (48% CP)	23.75	27.05	32.60	29.30	14.75
Corn DDGS	7.50	15.00	20.00	25.00	---
Nursery supplement	15.75	10.00	---	---	---
Limestone	0.70	0.95	1.05	1.28	0.70
Monocalcium phosphate (22% P)	0.84	0.83	0.60	0.65	0.15
Sodium chloride	0.35	0.38	0.26	0.31	0.53
Vitamin and mineral premix	0.08	0.10	0.15	0.15	0.10
L-Lys HCl	0.55	0.55	0.46	0.49	0.35
L-Thr	0.20	0.18	0.12	0.12	0.12
L-Trp	0.07	0.07	0.05	0.05	0.02
DL-Met	0.07	0.10	0.17	0.14	0.08
Choline chloride	0.01	---	---	---	---
Beef tallow	1.95	2.95	4.45	3.60	3.85
Phytase <sup>1</sup>	0.04	0.04	0.02	0.02	-
AV-E Digest <sup>2</sup>	5.00	2.50	2.50	-	-
XFE Liquid Energy <sup>3</sup>	---	---	0.50	0.50	0.25
Tri-basic copper chloride	---	0.03	---	---	---
Lipinate <sup>4</sup>	---	---	---	---	0.10
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Standardized ileal digestible (SID) AA, %					
Lys	1.40	1.40	1.41	1.32	0.81
Ile:Lys	57	58	62	62	56
Met and Cys:Lys	58	58	58	58	60
Thr:Lys	63	63	62	62	66
Trp:Lys	20	20	20	20	18
Val:Lys	67	67	68	68	66
Total Lys, %	1.56	1.56	1.58	1.48	0.89
CP, %	22.10	22.78	24.18	22.84	12.45
ME, kcal/lb	1,515	1,515	1,543	1,517	1,567
NE, kcal/lb	1,041	1,082	1,120	1,130	1,230
SID Lys:ME, g/Mcal	4.02	4.04	4.00	3.80	2.05
Ca, %	0.78	0.78	0.75	0.75	0.37
P, %	0.71	0.71	0.68	0.68	0.34
Available P, %	0.43	0.43	0.45	0.45	0.19

<sup>1</sup> Ronozyme HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ).

<sup>2</sup> AV-E Digest (XFE Products, Des Moines, IA).

<sup>3</sup> Liquid Energy (XFE Products, Des Moines, IA).

<sup>4</sup> Lipinate (Nutriquest LLC, Mason City, IA).

**Table 2. Feed budgets per pig averaged within treatments**

Phase	Control	Blended diets <sup>1</sup>		
		Phase 2	Phase 3	Phase 4
Phase 1	5.47 lb	5.47 lb	5.47 lb	5.47 lb
Phase 2	8.07 lb	2.75 lb late finishing feed, 5.5 lb 50:50% blend, 5.5 lb standard Phase 2	8.07 lb	8.07 lb
Phase 3	8.07 lb	8.07 lb	2.75 lb late finishing feed, 5.5 lb 50:50% blend, 5.5 lb standard Phase 3 <sup>2</sup>	8.07 lb
Phase 4	21 lb	21 lb	21 lb	2.75 lb late finishing feed, 5.5 lb 50:50% blend, 5.5 lb standard Phase 4
Phase 5	21 lb	15.5 lb	15.5 lb	15.5 lb

<sup>1</sup> Finishing feed was blended with standard nursery diets in different phases; blended diets were delivered in the sequence of finishing feed, 50% finishing and 50% standard blended diet, and standard diet.

<sup>2</sup> Three pens received the blended diets in the order of 50% finishing and 50% standard blended diet, finishing feed, and standard diet due to mistake.

**Table 3. Analyzed nutrient composition of experimental diets<sup>1</sup>**

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Finishing	50% Phase 2: 50% finishing blend	50% Phase 3: 50% finishing blend	50% Phase 4: 50% finishing blend
DM, %	89.2	89.6	89.1	88.5	87.2	87.8	88.5	88.7	87.7
CP, %	22.3	23.8	23.8	24.5	19.1	13.6	19.2	18.5	18.8
Ca, %	1.02	1.01	0.95	0.96	0.87	0.62	0.80	0.87	0.79
P, %	0.71	0.88	0.70	0.70	0.52	0.31	0.53	0.54	0.49
Zn, ppm	2,335	3,466	1,733	151	117	114	1,529	821	137
Cu, ppm	88	209	246	186	141	155	219	184	185

<sup>1</sup> Multiple samples of each diet were collected, blended and subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE).

**Table 4. Effects of blending finishing feed into different phases of nursery diets on growth performance<sup>1</sup>**

	Control	Blended diets <sup>2</sup>			SEM	P value
		Phase 2	Phase 3	Phase 4		
BW, lb						
d 0	12.8	12.9	12.9	12.9	0.112	0.976
d 7	15.5	15.6	15.5	15.5	0.153	0.962
d 14	21.6 <sup>a</sup>	20.6 <sup>b</sup>	21.8 <sup>a</sup>	21.9 <sup>a</sup>	0.294	<0.001
d 21	28.1 <sup>a</sup>	26.8 <sup>b</sup>	27.2 <sup>b</sup>	28.3 <sup>a</sup>	0.347	<0.001
d 28	35.7 <sup>a</sup>	34.2 <sup>b</sup>	34.2 <sup>b</sup>	35.3 <sup>a</sup>	0.369	0.001
d 35	45.8 <sup>a</sup>	43.7 <sup>c</sup>	44.3 <sup>bc</sup>	45.3 <sup>ab</sup>	0.484	0.003
d 47	66.2 <sup>a</sup>	64.1 <sup>b</sup>	64.8 <sup>ab</sup>	65.9 <sup>a</sup>	0.565	0.018
d 0 to 7						
ADG, lb	0.38	0.39	0.37	0.38	0.019	0.880
ADFI, lb	0.38	0.36	0.38	0.39	0.014	0.369
F/G	1.00	0.96	1.03	1.07	0.046	0.277
d 7 to 14						
ADG, lb	0.88 <sup>a</sup>	0.73 <sup>b</sup>	0.89 <sup>a</sup>	0.91 <sup>a</sup>	0.026	<0.001
ADFI, lb	0.99 <sup>a</sup>	0.91 <sup>b</sup>	0.98 <sup>a</sup>	1.01 <sup>a</sup>	0.029	0.002
F/G	1.13 <sup>a</sup>	1.25 <sup>b</sup>	1.11 <sup>a</sup>	1.11 <sup>a</sup>	0.021	<0.001
d 14 to 21						
ADG, lb	0.91 <sup>a</sup>	0.89 <sup>a</sup>	0.76 <sup>b</sup>	0.90 <sup>a</sup>	0.024	<0.001
ADFI, lb	1.23	1.23	1.23	1.23	0.024	0.991
F/G	1.35 <sup>a</sup>	1.39 <sup>a</sup>	1.62 <sup>b</sup>	1.38 <sup>a</sup>	0.033	<0.001
d 21 to 28						
ADG, lb	1.10 <sup>a</sup>	1.05 <sup>ab</sup>	1.03 <sup>b</sup>	1.00 <sup>b</sup>	0.018	0.003
ADFI, lb	1.44 <sup>ab</sup>	1.44 <sup>ab</sup>	1.39 <sup>b</sup>	1.48 <sup>a</sup>	0.021	0.018
F/G	1.32 <sup>a</sup>	1.38 <sup>b</sup>	1.35 <sup>ab</sup>	1.49 <sup>c</sup>	0.019	<0.001
d 28 to 35						
ADG, lb	1.43 <sup>a</sup>	1.36 <sup>b</sup>	1.43 <sup>a</sup>	1.43 <sup>a</sup>	0.023	0.067
ADFI, lb	1.95	1.91	1.95	2.01	0.035	0.236
F/G	1.37	1.41	1.36	1.41	0.020	0.174
d 35 to 47						
ADG, lb	1.70	1.69	1.72	1.71	0.018	0.644
ADFI, lb	2.86 <sup>a</sup>	2.75 <sup>b</sup>	2.76 <sup>b</sup>	2.81 <sup>ab</sup>	0.034	0.048
F/G	1.69 <sup>a</sup>	1.62 <sup>bc</sup>	1.61 <sup>c</sup>	1.64 <sup>b</sup>	0.012	<0.001
d 0 to 47						
ADG, lb	1.13 <sup>a</sup>	1.09 <sup>b</sup>	1.11 <sup>ab</sup>	1.12 <sup>a</sup>	0.012	0.031
ADFI, lb	1.62 <sup>a</sup>	1.57 <sup>b</sup>	1.59 <sup>ab</sup>	1.63 <sup>a</sup>	0.018	0.045
F/G	1.43	1.44	1.43	1.45	0.007	0.140

<sup>1</sup> A total of 1,260 weaned pigs (PIC TR4 × (Fast LW × PIC L02) with initial BW of 12.9 lb were used in a 47-d growth trial with 21 pigs per pen and 15 replications (pen) per treatment.

<sup>2</sup> Approximately 5.5 lb/pig of late finishing feed was blended with standard nursery diets at the beginning of different phases (as feed budgets presented in Table 2).

<sup>abc</sup> Means with different superscripts within a row differ ( $P < 0.05$ ).

**Table 5. Effects of blending finishing feed into different phases of nursery diets on production economics<sup>1</sup>**

Item	Control	Blended diets <sup>2</sup>			SEM	P value
		Phase 2	Phase 3	Phase 4		
Economics, \$/pig						
Feed cost <sup>3</sup>	12.37 <sup>a</sup>	11.74 <sup>b</sup>	12.01 <sup>b</sup>	12.39 <sup>a</sup>	0.134	<0.001
Gain value <sup>4</sup>	31.95 <sup>a</sup>	30.64 <sup>b</sup>	31.18 <sup>ab</sup>	31.64 <sup>a</sup>	0.334	0.031
Feed cost/lb gain <sup>5</sup>	0.232	0.231	0.230	0.234	0.0020	0.410
IOFC <sup>6</sup>	19.58	18.89	19.16	19.26	0.261	0.317

<sup>1</sup> A total of 1,260 weaned pigs (PIC TR4 × (Fast LW × PIC L02) with initial BW of 12.9 lb were used in a 47-d growth trial with 21 pigs per pen and 15 replications (pen) per treatment.

<sup>2</sup> Approximately 5.5 lb/pig of late finishing feed was blended with standard nursery diets at the beginning of different phases (as feed budgets presented in Table 2).

<sup>3</sup> Feed cost = diet cost × feed consumption.

<sup>4</sup> Gain value = total BW gain × \$0.60/lb.

<sup>5</sup> Feed cost per pound of gain = feed cost / (ADG × period length, d).

<sup>6</sup> Income over feed cost = gain value – feed cost.

<sup>ab</sup> Means with different superscripts within a row differ ( $P < 0.05$ ).

## Diet Formulation Method Influences the Response to Increasing Net Energy for Growing-Finishing Pigs

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### Summary

The objective of this study was to compare the effects of increasing dietary net energy (NE) in growing-finishing diets with maintaining a standardized ileal digestible (SID) Lys:NE ratio or not adjusting this ratio and keeping SID Lysine (Lys) constant across increasing NE density. A total of 150 pigs (Line 600 Duroc × Line 241, DNA, Columbus, NE) were used in a 91-d trial. Pens of pigs were blocked by gender and BW before being randomly assigned to treatments with 2 pigs per pen and 15 pens per treatment. Treatment diets included a low-energy negative control diet and a 2 × 2 factorial arrangement of treatments with main effects of increasing dietary NE (medium vs. high) and formulation method (constant SID Lys:NE ratio vs. constant percentage SID Lys). Increasing NE increased (linear,  $P = 0.001$ ) daily NE intake and improved (linear,  $P < 0.02$ ) F/G with both formulation methods; however, ADG and HCW only increased (linear,  $P < 0.03$ ) when a constant SID Lys:NE ratio was maintained as dietary NE increased. These results demonstrate the importance of maintaining a constant Lys:NE ratio when changing the NE of the diet for growing pigs.

Key words: calorie:lysine ratio, growing-finishing pig, lysine, net energy

### Introduction

Increasing dietary NE can improve growth rate and feed efficiency in growing-finishing pigs. Because increasing energy density usually decreases ADFI, pigs might not consume enough nutrients other than energy, such as AA. Therefore, to increase energy concentration and prevent a limited response in growth performance, diets could be adequate in other nutrients (Nitikanchana et al., 2015<sup>3</sup>). A previous trial<sup>4</sup> investigated the ef-

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<sup>3</sup> Nitikanchana, S., S. S. Dritz, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, and B. J. White. 2015. Regression analysis to predict growth performance from dietary energy in growing-finishing pigs. *J. Anim. Sic.* 93:2826-2839.

<sup>4</sup> Marçal, D. The effects of increasing energy content in diets for barrows, Ph.D. Dissertation, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil. Not yet published.

fects of increasing dietary NE without maintaining a SID Lys:NE ratio. In that study, increasing NE improved F/G, but there was no change in growth rate of growing-finishing pigs. Thus, the present study was conducted with the objective to compare the effects of increasing dietary NE with a constant SID Lys:NE ratio or a constant percentage SID Lys (no Lys:NE ratio) on growth performance and carcass characteristics of growing-finishing pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were housed in an environmentally controlled barn with completely slatted concrete floor. Each pen was equipped with a single-hole stainless steel feeder and a nipple drinker for ad libitum access to feed and water.

A total of 150 pigs, 70 barrows and 80 gilts (Line 600 Duroc × Line 241; DNA, Columbus, NE) were used in a 91-d trial. Pens of pigs were blocked by gender and BW and randomly assigned to diets with 2 pigs per pen and 15 pen per treatments (7 pens with barrows and 8 pens with gilts).

Treatments were arranged in a  $2 \times 2 + 1$  factorial including a low-energy control diet or diets with increasing NE (medium or high) and 2 formulation methods (constant SID Lys:NE ratio vs. constant percentage SID Lys).

All experimental diets were fed in meal form. Diets were formulated to be fed in 4 phases (65 to 110, 110 to 155, 155 to 220, and 220 to 280 lb)<sup>5</sup> and were prepared at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

The control low-NE diet was formulated to a 4.08, 3.50, 3.02, and 2.61 SID Lys:NE ratio and 0.91, 0.78, 0.66, and 0.57% SID Lys in Phases 1, 2, 3 and 4, respectively (Table 1). Two high-NE diets were formulated to achieve either the same SID Lys:NE ratios as the low-NE diet or same percentage SID Lys as the low-NE diet. The low-NE diet was blended with each of the high-NE diets in a 50:50 ratio to obtain the 2 medium-NE diets. Soybean hulls were used in the low-NE diet and choice white grease was used in the high-NE diets. Crystalline AA also were used to achieve the constant SID Lys:NE ratio vs. constant percentage SID Lys diets. Thus, this study was composed of 5 dietary treatments (low-NE; medium-NE with constant SID Lys:NE ratio; high-NE with constant SID Lys:NE ratio; medium-NE with constant percentage SID Lys; and high-NE with constant percentage SID Lys. For diet formulation, feed ingredients were assigned an NE value taken from INRA (2004).<sup>6</sup>

<sup>5</sup> NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

<sup>6</sup> INRA (Institut National de la Recherche Agronomique). 2004. Tables of composition and nutritional value of feed materials, Sauvant, D., J-M. Perez, and G. Tran, Eds. Wageningen Academic Publishers, The Netherlands and INRA, Paris, France.

Pigs were weighed and feed disappearance was recorded on d 0, 19, 34, 61, and 91 to determine ADG, ADFI, and F/G. To analyze the data the study was divided in three phases of approximate equal time (Phase 1, d 0 to 34; Phase 2, d 34 to d 61; and Phase 3, d 61 to 91).

The NE intake and SID Lys intake were determined by multiplying the total feed intake  $\times$  NE or SID Lys content in the diet and divided by days in the period. Caloric and SID Lys efficiencies were determined by dividing total NE intake or total SID Lys intake by the total gain in each period.

On d 91, all pigs were individually weighed and tattooed with a unique identifier. Pigs were transported to a commercial harvesting facility (Triumph Foods LLC, St. Joseph, MO) and held in lairage overnight prior to processing and carcass data collection. At the plant, HCW, backfat depth, loin depth and jowl IV value were collected. Percentage carcass yield was calculated by dividing individual HCW obtained at the packing plant by the individual final live weight obtained at the farm.

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) with dietary treatment as fixed effect. Block was included in the model as a random effect. Pen was the experimental unit for all data analysis. For analysis of backfat depth, loin depth, and percentage lean, HCW was used as a covariate. The main effect of formulation method was tested and contrast coefficients were used to evaluate linear and quadratic responses to dietary NE level within SID Lys formulation. Significance were set at  $P < 0.05$  and tendencies were set at  $P \leq 0.10$ .

## Results and Discussion

From d 0 to 34, increasing dietary NE increased daily NE intake (linear,  $P < 0.001$ ) with both formulation methods (Table 5); however, daily SID Lys intake increased (linear,  $P < 0.001$ ) only when SID Lys:NE ratio was kept constant as dietary NE increased. Average daily gain increased (linear,  $P = 0.037$ ) as did BW (linear,  $P = 0.005$ ) as energy concentration was increased with constant SID Lys:NE ratio. Pigs fed constant SID Lys:NE ratio diets had improved (linear,  $P = 0.009$ ) F/G with increasing dietary NE. Moreover, F/G was better ( $P = 0.026$ ) in pigs fed diets in which the SID Lys:NE ratio remained constant compared those fed a constant percentage SID Lys as NE increased. The efficiency of NE utilization worsened (linear,  $P < 0.001$ ) with both formulation methods as dietary NE increased suggesting the NE level of soybean hulls may have been underestimated or NE level of fat may have been overestimated in diet formulation. As a result, efficiency of Lys utilization also worsened (linear,  $P < 0.001$ ) as dietary NE increased when a constant Lys:NE ratio was maintained.

The responses from d 34 to 61 were similar to those from d 0 to 34. Daily NE intake increased (linear,  $P < 0.02$ ) with increasing dietary NE for both formulation methods; however, SID Lys intake only increased (linear,  $P < 0.001$ ) when SID Lys:NE ratio was kept constant as dietary NE increased. As a result, ADG and BW increased (linear,  $P < 0.006$ ) as dietary NE increased when maintaining constant SID Lys:NE ratio. In this phase, F/G improved (linear,  $P < 0.03$ ) by increasing dietary NE with both formulation methods. However, NE efficiency tended to be poorer (linear,  $P = 0.055$ ) by

increasing dietary NE with a constant percentage SID Lys, but was not affected when a constant SID Lys:NE was maintained.

The improvements in growth performance to increasing dietary NE were much less from d 61 to 91 than in earlier phases. However similar to earlier phases, pigs had greater (linear,  $P < 0.048$ ) NE intake as dietary NE increased with both formulation methods, but SID Lys intake only increased (linear,  $P = 0.001$ ) when SID Lys:NE ratio was maintained. In this last phase, despite the response observed in NE and Lys intake, there was no effect of dietary NE on ADG or F/G. Like previous phases, increased NE intake resulted in poor NE efficiency and increased SID Lys intake resulted in poorer SID Lys efficiency.

Overall, increasing dietary NE increased (linear,  $P = 0.022$ ) ADG only in pigs fed with diets with a constant SID Lys:NE ratio. However, F/G was improved ( $P < 0.017$ ) with both formulation methodologies. No effect of energy level was observed for ADFI, which resulted in an increase (linear,  $P = 0.001$ ) in NE intake as dietary NE increased with both formulation methods. In treatments in which the SID Lys:NE ratio was kept constant, the increase observed in NE intake also resulted in increased (linear,  $P < 0.001$ ) SID Lys intake. Thus, as pigs had more NE and SID Lys intake they were less efficient in utilization of the energy and SID Lys.

For carcass characteristics (Table 6), HCW of pigs fed with a constant SID Lys:NE ratio were heavier ( $P = 0.027$ ) than carcass of pigs fed with constant percentage SID Lys. Furthermore, increasing dietary NE within the SID Lys:NE ratio diets increased (linear,  $P = 0.002$ ) HCW. Carcass yield increased (linear,  $P < 0.03$ ) by increasing dietary NE with both formulation methods. A tendency for a dietary NE  $\times$  SID Lys formulation interaction was observed for backfat thickness. Maintaining a constant percentage SID Lys as dietary NE increased resulted in increased (quadratic,  $P = 0.009$ ) backfat depth compared with maintaining a constant SID Lys:NE ratio. Loin depth tended to be less in pigs fed constant SID Lys:NE ratio than in pigs fed diets with constant percentage SID Lys ( $P = 0.098$ ). Increasing dietary NE with constant percentage SID Lys also tended (linear,  $P = 0.099$ ) to increase loin depth and had a mixed effect (quadratic,  $P = 0.015$ ) on fat-free lean. Increasing dietary NE increased (linear,  $P < 0.04$ ) jowl IV with both formulation methods as expected due to increasing added dietary fat in those diets. Salyer et al. (2012<sup>7</sup>) also observed increased jowl IV by increasing the amount of choice white grease in finishing pig diets.

In summary, increasing dietary NE with a constant SID Lys:NE ratio increased AA intake and resulted in improvements in ADG and F/G whereas increasing energy without keeping a constant SID Lys:NE ratio improved only F/G. Although pigs fed the low-NE diet grew slower, they were more efficient at utilizing the NE and SID Lys that they consumed. Increasing energy concentration without keeping SID Lys:NE constant also increased backfat depth.

<sup>7</sup> Salyer, J.A., J.M. DeRouchey, M.D. Tokach, S.S. Dritz, R.D. Goodband, J.L. Nelssen, and D.B. Petry. 2012. Effects of dietary wheat middlings, distillers dried grains with solubles, and choice white grease on growth performance, carcass characteristics, and carcass fat quality of finishing pigs. *J. Anim. Sci.* 90:2620–2630.

**Table 1. Diet composition of Phase 1 (as fed-basis)<sup>1</sup>**

Item	NE level:	Formulation method				
		Control	Constant Lys:NE		Constant Lys %	
		Low	Medium	High	Medium	High
Ingredient, %						
Corn		68.87	68.36	67.86	71.46	74.05
Soybean meal (45% CP)		19.51	22.72	25.93	19.96	20.41
Soybean hulls		8.67	4.33	---	4.33	---
Choice white grease		---	1.60	3.19	1.25	2.51
Monocalcium phosphate (21% P)		0.90	0.88	0.85	0.89	0.88
Limestone		0.90	0.95	1.00	0.96	1.03
Sodium chloride		0.35	0.35	0.35	0.35	0.35
L-Lys-HCl		0.30	0.30	0.30	0.30	0.30
DL-Met		0.07	0.08	0.10	0.07	0.06
L-Thr		0.09	0.09	0.09	0.09	0.08
L-Trp		0.02	0.01	0.01	0.02	0.02
L-Val		0.01	0.01	0.01	0.01	0.01
Trace mineral premix		0.15	0.15	0.15	0.15	0.15
Vitamin premix		0.15	0.15	0.15	0.15	0.15
Phytase <sup>2</sup>		0.02	0.02	0.02	0.02	0.02
Calculated analysis						
Standardized ileal digestible (SID) AA, %						
Lys		0.91	0.98	1.05	0.91	0.91
Ile:Lys		61	61	62	61	61
Leu:Lys		136	135	133	138	139
Met:Lys		33	33	34	33	32
Met and Cys:Lys		58	58	58	58	58
Thr:Lys		62	62	62	62	62
Trp:Lys		19	19	19	19	19
Val:Lys		69	69	69	69	69
Total Lys, %		1.04	1.11	1.17	1.03	1.03
CP, %		16.3	17.3	18.4	16.3	16.2
ME, kcal/lb		1,438	1,499	1,560	1,493	1,547
NE, kcal/lb <sup>3</sup>		1,013	1,087	1,162	1,088	1,163
SID Lys:ME, g/Mcal		2.87	2.96	3.04	2.77	2.67
SID Lys:NE, g/Mcal		4.08	4.08	4.08	3.80	3.55
Ca, %		0.60	0.60	0.60	0.60	0.60
P, %		0.52	0.53	0.54	0.52	0.53
Available P, %		0.34	0.34	0.34	0.34	0.34

<sup>1</sup> Phase 1 experimental diets were fed from d 0 to 19 (78- to 120-lb BW).

<sup>2</sup> Ronozyme Hiphos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>3</sup> NE values from ingredients were obtained from INRA (2004).

**Table 2. Diet composition of Phase 2 (as fed-basis)<sup>1</sup>**

Item	NE level:	Formulation method				
		Control	Constant Lys:NE		Constant Lys %	
		Low	Medium	High	Medium	High
Ingredient, %						
Corn		72.55	73.82	75.10	76.64	80.73
Soybean meal (45% CP)		13.76	16.78	19.80	14.27	14.78
Soybean hulls		10.98	5.49	---	5.49	---
Choice white grease		---	1.15	2.30	0.84	1.68
Monocalcium phosphate (21% P)		0.75	0.73	0.70	0.73	0.70
Limestone		0.85	0.91	0.98	0.94	1.03
Sodium chloride		0.35	0.35	0.35	0.35	0.35
L-Lys-HCl		0.30	0.30	0.30	0.30	0.30
DL-Met		0.04	0.05	0.06	0.04	0.03
L-Thr		0.08	0.08	0.08	0.08	0.07
L-Trp		0.02	0.02	0.02	0.02	0.02
L-Val		0.01	0.01	0.01	0.01	0.00
Trace mineral premix		0.15	0.15	0.15	0.15	0.15
Vitamin premix		0.15	0.15	0.15	0.15	0.15
Phytase <sup>2</sup>		0.02	0.02	0.02	0.02	0.02
Calculated analysis						
Standardized ileal digestible (SID) AA, %						
Lys		0.78	0.84	0.90	0.78	0.78
Ile:Lys		59	60	61	60	60
Leu:Lys		142	141	140	145	148
Met:Lys		32	32	32	31	31
Met and Cys:Lys		58	58	58	58	58
Thr:Lys		62	62	62	62	62
Trp:Lys		19	19	19	19	19
Val:Lys		69	69	69	69	69
Total Lys, %		0.90	0.95	1.01	0.89	0.88
CP, %		14.07	15.05	16.03	14.07	14.08
ME, kcal/lb		1,428	1,487	1,546	1,481	1,534
NE, kcal/lb <sup>3</sup>		1,004	1,084	1,164	1,085	1,165
SID Lys:ME, g/Mcal		2.46	2.55	2.63	2.37	2.29
SID Lys:NE, g/Mcal		3.50	3.50	3.50	3.24	3.02
Ca, %		0.55	0.55	0.55	0.55	0.55
P, %		0.46	0.47	0.49	0.46	0.47
Available P, %		0.30	0.30	0.30	0.30	0.30

<sup>1</sup> Phase 2 experimental diets were fed from d 20 to 34 (120- to 158-lb BW).

<sup>2</sup> Ronozyme Hiphos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>3</sup> NE values from ingredients were obtained from INRA (2004).

**Table 3. Diet composition of Phase 3 (as fed-basis)<sup>1</sup>**

Item	NE level:	Formulation method				
		Control	Constant Lys:NE		Constant Lys %	
		Low	Medium	High	Medium	High
Ingredient, %						
Corn		75.49	78.38	81.28	80.99	86.49
Soybean meal (45% CP)		8.98	11.86	14.74	9.55	10.12
Soybean hulls		13.14	6.57	---	6.57	---
Choice white grease		---	0.74	1.48	0.45	0.90
Monocalcium phosphate (21% P)		0.63	0.61	0.60	0.61	0.60
Limestone		0.70	0.78	0.85	0.79	0.88
Sodium chloride		0.35	0.35	0.35	0.35	0.35
L-Lys-HCl		0.30	0.30	0.30	0.30	0.30
DL-Met		0.02	0.03	0.03	0.01	0.01
L-Thr		0.09	0.09	0.09	0.08	0.08
L-Trp		0.03	0.02	0.02	0.03	0.02
L-Val		0.01	0.01	0.00	0.01	0.00
Trace mineral premix		0.13	0.13	0.13	0.13	0.13
Vitamin premix		0.13	0.13	0.13	0.13	0.13
Phytase <sup>2</sup>		0.02	0.02	0.02	0.02	0.02
Calculated analysis						
Standardized ileal digestible (SID) AA, %						
Lys		0.66	0.72	0.78	0.66	0.66
Ile:Lys		57	59	60	58	59
Leu:Lys		148	148	148	153	158
Met:Lys		30	31	31	30	29
Met and Cys:Lys		58	58	58	58	58
Thr:Lys		64	64	64	64	64
Trp:Lys		19	19	19	19	19
Val:Lys		69	69	70	70	70
Total Lys, %		0.78	0.83	0.88	0.76	0.75
CP, %		12.25	13.18	14.11	12.28	12.32
ME, kcal/lb		1,421	1,478	1,535	1,473	1,524
NE, kcal/lb <sup>3</sup>		996	1,081	1,165	1,081	1,167
SID Lys:ME, g/Mcal		2.12	2.21	2.29	2.04	1.97
SID Lys:NE, g/Mcal		3.02	3.02	3.02	2.78	2.58
Ca, %		0.47	0.47	0.47	0.47	0.47
P, %		0.41	0.43	0.45	0.42	0.43
Available P, %		0.27	0.27	0.27	0.27	0.27

<sup>1</sup> Phase 3 experimental diets were fed from d 35 to 61 (158- to 220-lb BW).

<sup>2</sup> Ronozyme Hiphos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>3</sup> NE values from ingredients were obtained from INRA (2004).

**Table 4. Diet composition of Phase 4 (as fed-basis)<sup>1</sup>**

Item	NE level:	Formulation method				
		Control	Constant Lys:NE		Constant Lys %	
			Low	Medium	High	Medium
Ingredient, %						
Corn		76.98	80.91	84.84	83.22	89.45
Soybean meal (45% CP)		6.68	9.34	12.00	7.28	7.88
Soybean hulls		14.30	7.15	---	7.15	---
Choice white grease		---	0.49	0.99	0.24	0.49
Monocalcium phosphate (21% P)		0.50	0.48	0.45	0.50	0.50
Limestone		0.63	0.71	0.80	0.71	0.80
Sodium chloride		0.35	0.35	0.35	0.35	0.35
L-Lys-HCl		0.25	0.25	0.25	0.25	0.25
DL-Met		0.02	0.03	0.03	0.01	0.00
L-Thr		0.07	0.07	0.07	0.06	0.06
L-Trp		0.02	0.02	0.02	0.02	0.02
L-Val		0.00	0.00	0.00	0.00	0.00
Trace mineral premix		0.10	0.10	0.10	0.10	0.10
Vitamin premix		0.10	0.10	0.10	0.10	0.10
Phytase <sup>2</sup>		0.02	0.02	0.02	0.02	0.02
Calculated analysis						
Standardized ileal digestible (SID) AA, %						
Lys		0.57	0.62	0.67	0.57	0.57
Ile:Lys		60	62	63	61	62
Leu:Lys		163	163	163	169	175
Met:Lys		33	34	34	32	32
Met and Cys:Lys		64	64	64	64	64
Thr:Lys		65	65	65	65	65
Trp:Lys		19	19	19	19	19
Val:Lys		73	73	74	74	76
Total Lys, %		0.68	0.72	0.76	0.67	0.65
CP, %		11.31	12.17	13.03	11.37	11.42
ME, kcal/lb		1,418	1,474	1,529	1,469	1,519
NE, kcal/lb <sup>3</sup>		991	1,079	1,166	1,079	1,167
SID Lys:ME, g/Mcal		1.82	1.91	1.99	1.76	1.70
SID Lys:NE, g/Mcal		2.61	2.61	2.61	2.40	2.21
Ca, %		0.42	0.42	0.42	0.42	0.42
P, %		0.37	0.39	0.40	0.38	0.40
Available P, %		0.24	0.24	0.24	0.24	0.24

<sup>1</sup> Phase 4 experimental diets were fed from d 62 to 91 (220- to 280-lb BW).

<sup>2</sup> Ronozyme Hiphos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>3</sup> NE values from ingredients were obtained from INRA (2004).

**Table 5. Effects of increasing dietary NE with constant standardized ileal digestible (SID Lys:NE ratio or constant percentage SID Lys) on growth performance of growing-finishing pigs<sup>1</sup>**

Item	NE level:	Formulation method					SEM	Probability, <i>P</i> <				
		Control		Constant Lys:NE		Constant Lys %		Lys:NE vs. Lys%	NE Lys:NE		NE Lys%	
		Low	Medium	High	Medium	High			Linear	Quadratic	Linear	Quadratic
BW, lb												
d 0		78.6	78.6	78.7	78.6	78.7	1.98	0.959	0.884	0.933	0.884	1.000
d 34		154.2	160.2	160.9	156.3	156.9	3.03	0.018	0.005	0.191	0.242	0.698
d 61		213.9	222.9	225.6	217.0	217.0	3.41	0.003	0.001	0.280	0.362	0.598
d 91		279.8	286.7	291.8	281.2	281.9	3.88	0.023	0.013	0.821	0.654	0.938
d 0 to 34												
ADG, lb		2.16	2.28	2.32	2.22	2.23	0.058	0.163	0.037	0.506	0.323	0.741
ADFI, lb		4.81	4.95	4.87	5.00	4.87	0.134	0.835	0.673	0.381	0.716	0.191
F/G		2.23	2.18	2.10	2.26	2.18	0.036	0.026	0.009	0.885	0.262	0.240
NE intake, kcal/d		4,874	5,386	5,664	5,445	5,662	147.5	0.797	<0.001	0.394	<0.001	0.199
SID Lys intake, g/d		18.5	20.5	21.5	19.2	18.7	0.541	<0.001	<0.001	0.346	0.662	0.203
NE efficiency		2,262	2,365	2,445	2,454	2,535	39.9	0.020	0.001	0.805	<0.001	0.229
SID Lys efficiency		8.56	8.99	9.30	8.66	8.37	0.149	<0.001	<0.001	0.734	0.348	0.264
d 34 to 61												
ADG, lb		2.18	2.32	2.40	2.25	2.23	0.056	0.025	0.006	0.591	0.531	0.490
ADFI, lb		6.95	7.23	6.94	7.16	6.60	0.182	0.236	0.957	0.184	0.148	0.072
F/G		3.20	3.12	2.91	3.20	2.97	0.075	0.300	0.006	0.484	0.030	0.184
NE intake, kcal/d		7,040	7,857	8,063	7,789	7,679	199.1	0.228	<0.001	0.185	0.018	0.064
SID Lys intake, g/d		20.8	23.6	24.5	21.4	19.76	0.571	<0.001	<0.001	0.165	0.166	0.085
NE efficiency		3,240	3,389	3,381	3,487	3,458	81.5	0.269	0.211	0.420	0.055	0.159
SID Lys efficiency		9.58	10.18	10.29	9.59	8.90	0.232	<0.001	0.027	0.380	0.035	0.199

*continued*

**Table 5. Effects of increasing dietary NE with constant standardized ileal digestible (SID Lys:NE ratio or constant percentage SID Lys) on growth performance of growing-finishing pigs<sup>1</sup>**

Item	NE level:	Formulation method					SEM	Probability, <i>P</i> <				
		Control	Constant Lys:NE		Constant Lys %			Lys:NE vs. Lys%	NE Lys:NE		NE Lys%	
		Low	Medium	High	Medium	High			Linear	Quadratic	Linear	Quadratic
d 61 to 91												
ADG, lb		2.20	2.13	2.21	2.14	2.17	0.061	0.795	0.904	0.272	0.677	0.550
ADFI, lb		7.00	7.23	6.72	6.92	6.58	0.186	0.206	0.270	0.090	0.100	0.546
F/G		3.20	3.41	3.07	3.23	3.04	0.071	0.147	0.207	0.002	0.120	0.195
NE intake, kcal/d		7,086	7,865	7,804	7,528	7,651	206.4	0.220	0.013	0.088	0.048	0.511
SID Lys intake, g/d		18.1	20.4	20.4	17.9	17.0	0.512	<0.001	0.001	0.075	0.122	0.571
NE efficiency		3,240	3,711	3,567	3,519	3,537	78.5	0.161	0.005	0.002	0.010	0.178
SID Lys efficiency		8.27	9.60	9.33	8.36	7.86	0.198	<0.001	<0.001	0.002	0.145	0.225
d 0 to 91												
ADG, lb		2.17	2.24	2.30	2.20	2.21	0.039	0.108	0.022	0.967	0.487	0.817
ADFI, lb		6.13	6.31	6.06	6.26	5.93	0.138	0.491	0.672	0.161	0.270	0.144
F/G		2.82	2.82	2.64	2.84	2.68	0.041	0.412	0.002	0.060	0.017	0.070
NE intake, kcal/d		6,209	6,864	7,036	6,812	6,902	152.6	0.509	<0.001	0.161	0.001	0.137
SID Lys intake, g/d		19.0	21.3	22.0	19.4	18.46	0.446	<0.001	<0.001	0.110	0.326	0.151
NE efficiency		2,858	3,070	3,064	3,093	3,123	45.3	0.354	0.001	0.047	<0.001	0.059
SID Lys efficiency		8.75	9.52	9.59	8.83	8.35	0.134	<0.001	<0.001	0.023	0.027	0.073

<sup>1</sup> A total of 150 pigs (Line 600 × Line 241, DNA, Columbus, NE) were used in a 91-d growing-finishing trial with 2 pigs per pen and 15 pens per treatment.

**Table 6. Effects of increasing dietary NE with constant standardized ileal digestible (SID Lys:NE ratio or constant percentage SID Lys) on carcass characteristics of growing-finishing pigs<sup>1</sup>**

Item	NE level:	Formulation method					SEM	Probability, <i>P</i> <				
		Control	Constant Lys:NE		Constant Lys%			Lys:NE vs. Lys%	NE Lys:NE		NE Lys%	
		Low	Medium	High	Medium	High			Linear	Quadratic	Linear	Quadratic
HCW, lb		205.3	212.1	217.9	207.2	210.5	3.37	0.027	0.002	0.876	0.188	0.827
Carcass yield, %		73.4	74.0	74.7	73.6	74.6	0.40	0.664	0.024	0.925	0.027	0.466
Backfat, cm		19.0	18.6	18.8	20.7	19.0	0.58	0.041	0.872	0.687	0.952	0.009
Loin depth, cm		61.7	60.1	62.5	62.1	64.5	1.20	0.098	0.633	0.166	0.099	0.522
Fat-free lean, %		53.0	52.9	53.3	52.5	53.6	0.28	0.935	0.541	0.458	0.121	0.015
Jowl iodine value		67.5	67.8	68.6	67.1	69.3	0.38	0.979	0.034	0.526	0.001	0.006

<sup>1</sup> A total of 150 pigs (Line 600 × Line 241 DNA, Columbus, NE) were used in a 91-d growing-finishing trial with 2 pigs per pen and 15 pens per treatment.

## Effects of Crude Protein and Amino Acid to Lysine Ratio on Finishing Pig Growth Performance and Carcass Characteristics<sup>1,2</sup>

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### Summary

The increased availability of synthetic amino acids has reduced the amount of intact protein sources used in swine diets. The objective of this study was to determine the effects of different CP levels and AA to Lys ratios on growth performance and carcass characteristics in late finishing pigs. A total of 1,682 pigs (327 × 1050, PIC, Hendersonville, TN; initially 252.7 lb BW) were used in a 25-d growth trial arranged in an unbalanced randomized complete block design with 25 pigs per pen and initially 8 or 16 pens per treatment. Dietary treatments were arranged in a 2 × 2 + 1 factorial consisting of combinations of 10.3 or 13.5% CP and 2 AA to Lys ratios plus a control diet (13.5% CP from soybean meal). The standardized ileal digestible (SID) ratios to Lys were 55% Met+Cys, 68% Thr, 17% Trp, 65% Val, 56% Ile, and 32% His for PIC (2013)<sup>5</sup> and 60% Met+Cys, 68% Thr, 20% Trp, 72% Val, 55% Ile, and 37% His for the Modified ratio.

Overall, from d 0 to 25, pigs fed the control diet had increased ADG ( $P < 0.001$ ) compared with pigs fed diets formulated with the PIC or Modified AA:Lys ratios. There were no statistical differences in ADFI observed between the treatments. For F/G, there was a 2-way marginally significant interaction ( $P = 0.066$ ) where F/G was improved for pigs fed the PIC AA:Lys ratios with 13.5% CP compared to those fed diets with 10.3% CP; however, there were no statistical differences in F/G between CP levels in pigs fed Modified AA:Lys ratios. Final BW was increased in pigs fed the control compared to pigs fed diets formulated with the PIC ( $P = 0.017$ ) or Modified ( $P < 0.001$ ) AA:Lys ratios. Pigs fed 10.5% CP provided by glutamic acid and glycine, regardless of AA:Lys ratio, had increased ( $P = 0.031$ ) carcass yield; however, there was

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<sup>4</sup> Ajinomoto Heartland Inc., Chicago, IL.

<sup>5</sup> PIC. 2013. Nutrient specifications manual. p. 56. accessed on December 8th, 2015 at <http://na.picgenus.com/resources.aspx>

no statistical differences between the dietary treatments regarding HCW, backfat, loin depth, and percentage lean.

In conclusion, reducing intact protein (soybean meal) decreased growth performance and the inclusion of a nitrogen source (glycine and glutamic acid) was not able to recover growth performance in this commercial study. The 2 amino acid ratios in the low crude protein diets evaluated in this study did not improve growth performance or carcass characteristics.

Key words: amino acid ratio, crude protein level, growth, finishing pig

## Introduction

The increased availability of synthetic amino acids has reduced the levels of intact protein sources used in swine diets. Thus, the amount of nitrogen available for synthesis of non-essential AA has decreased. It has been observed that AA supplied by intact protein sources can be replaced by synthetic AA to meet the requirement of the first 5 limiting AA; however, including synthetic AA beyond the first 5 limiting has been shown to have inconsistent results, especially with pigs in the late finishing phase.<sup>6,7</sup>

The objective of this study was to determine the effects of CP level and AA to Lys ratios on growth performance and carcass characteristics of late finishing pigs. The hypotheses were: 1) reducing intact protein would impair growth performance but the inclusion of a nitrogen source would recover performance, and 2) increased AA ratios would improve growth performance.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee (IACUC) approved the protocol used in this experiment. The experiment was conducted at a commercial research facility in Minnesota. The barn was naturally ventilated and double-curtain-sided and pens had completely slatted flooring and deep pits for manure storage. Each pen was equipped with a 3-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer for ad libitum intake for feed and water. The facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions.

Five representative samples of corn, soybean meal, and dried distillers grains with solubles (DDGS) were collected each week for 5 wk and analyzed in duplicate for total amino acids and CP (Ajinomoto Heartland, Inc., Chicago, IL). These values were then used in diet formulation. Other nutrients and SID AA digestibility coefficients used for diet formulation were obtained from NRC (2012).

<sup>6</sup> Gloaguen, M., L. Floc'h, E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. *J. Anim. Sci.* 92:637-644.

<sup>7</sup> Apple, J. K., C. V. Maxwell, T. C. Tsai, H. J. Kim, D. G. Cook, K. J. Touchette, J. E. Thomson, J. Less, J. J. Chewing. Effect of feed-grade amino acid supplementation in reduced crude protein (RCP) diets formulated on a NE basis on performance and carcass characteristic of growing-finishing pigs. *J. Anim. Sci.* 93:19 (Abstr.).

A total of 1,682 pigs (327 × 1050, PIC, Hendersonville, TN; initially 252.7 lb BW) were used in a 25-d growth trial arranged in an unbalanced randomized complete block design with 25 pigs per pen and initially 8 or 16 pens per treatment.

Dietary treatments consisted of combinations CP (10.3 or 13.5%) and 2 AA to Lys ratios (“PIC” or “Modified”) in a 2 × 2 + 1 factorial arrangement where the control diet was formulated at 13.5% CP using soybean meal as the major protein source. Crude protein was increased from 10.3 to 13.5% by inclusion of glutamic acid and glycine at a 2:1 ratio with the exception of the positive control diet where soybean meal was increased to achieve the desired CP level. The SID ratios to Lys were 55% Met+Cys, 68% Thr, 17% Trp, 65% Val, 56% Ile, and 32% His for PIC (2013)<sup>5</sup> and 60% Met+Cys, 68% Thr, 20% Trp, 72% Val, 55% Ile, and 37% His for the Modified treatments. Diets were fed in meal form and were corn-soybean meal-based with 10% DDGS (Table 1).

Pens of pigs were weighed and feed disappearance measured at the beginning, d 13, 21, and 25 to determine ADG, ADFI, and F/G. Prior to marketing, the pigs were individually tattooed with a pen ID number to allow for carcass measurements to be recorded on a pen basis. Carcass measurements taken at the plant (JBS Swift and Company, Worthington, MN) included HCW, loin depth, backfat, and percentage lean. Percentage carcass yield was calculated by dividing the individual HCW at the plant by the pig’s pen average final live weight at the farm. A total of 4 pens, one from each treatment (except treatment 13.5% CP and PIC ratio) was removed due to feeder flow ability issues.

Responses measured at the pen level were analyzed using a general linear mixed model and contrasts were used to evaluate the effect of the different factors on ADG, ADFI, F/G, and BW. Heterogeneous residual variances as a function of the response variables were fitted as needed. Model assumptions were checked and considered to be appropriately met. The experimental data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Hot carcass weight served as a covariate for the analysis of backfat, loin depth, and lean percentage. Results were considered significant at  $P \leq 0.05$  and a tendency at  $P \leq 0.10$ .

## Results and Discussion

From d 0 to 25, ADG was increased in pigs fed the control diet compared to PIC ( $P < 0.001$ ) and Modified ( $P < 0.001$ ) AA:Lys ratios. There were no statistical differences in ADFI between the treatments. For F/G, there was a 2-way marginally significant interaction ( $P = 0.066$ ) where F/G was improved in the PIC AA:Lys ratio with 13.5% compared to 10.3%; however, no statistical differences in F/G between CP levels in pigs fed Modified AA:Lys ratio were observed. Final BW was increased in pigs fed the control diet compared to PIC ( $P = 0.017$ ) and Modified ( $P < 0.001$ ) AA:Lys ratios. Pigs fed 10.5% CP provided by glutamic acid and glycine, regardless of AA:Lys ratio, had increased ( $P = 0.031$ ) carcass yield (Table 3); however, there were no statistical differences between the dietary treatments regarding HCW, backfat, loin depth, and percentage lean.

In conclusion, reducing intact protein impaired growth performance, and the inclusion of a nitrogen source was not able to recover growth performance in this commercial

study. This could be related to dietary electrolyte balance, other amino acids, or growth factors provided by soybean meal that were not available in the diets with 13.5% CP with added L-Glu or Gly. Increasing AA ratios relative to Lys did not improve growth performance or carcass characteristics.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Control diet	PIC ratios, CP,%		Modified ratios, CP, %	
		13.5	10.3	13.5	10.3
Ingredient					
Corn	77.46	81.32	85.96	81.19	85.82
Soybean meal (46% CP)	10.32	1.44	1.15	1.44	1.16
DDGS <sup>1</sup>	10.00	10.00	10.00	10.00	10.00
Choice white grease	0.50	0.50	0.50	0.50	0.50
Calcium carbonate	0.85	0.85	0.85	0.85	0.85
Dicalcium phosphate (18.5% P)	0.10	0.15	0.15	0.15	0.15
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys-HCl	0.215	0.513	0.513	0.513	0.513
DL-Met	---	0.025	0.025	0.055	0.055
L-Thr	0.040	0.180	0.180	0.180	0.180
L-Trp	0.014	0.045	0.045	0.065	0.065
L-Val	---	0.055	0.055	0.100	0.100
L-Ile	---	0.075	0.075	0.070	0.070
L-Glu	---	2.900	---	2.900	---
Gly	---	1.450	---	1.450	---
L-His	---	---	---	0.035	0.035
Vitamin-mineral premix	0.100	0.100	0.100	0.100	0.100
Phytase <sup>2</sup>	0.050	0.050	0.050	0.050	0.050
Total	100.0	100.0	100.0	100.0	100.0

*continued*

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Control diet	PIC ratios, CP,%		Modified ratios, CP, %	
		13.5	10.3	13.5	10.3
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	0.63	0.63	0.63	0.63	0.63
Ile:Lys	70	56	57	55	56
Leu:Lys	189	148	152	148	152
Met:Lys	32	28	29	33	34
Met and Cys:Lys	66	55	56	60	61
Thr:Lys	68	68	69	68	69
Trp:Lys	20.0	17.0	17.0	20.1	20.2
Val:Lys	81	65	66	72	73
His:Lys	47	32	32	37	38
ME, kcal/lb	1,525	1,528	1,532	1,529	1,532
NE NRC, kcal/lb	1,163	1,185	1,190	1,185	1,190
SID Lys:NE, g/Mcal	2.46	2.41	2.40	2.41	2.40
SID Lys:ME, g/Mcal	1.87	1.87	1.87	1.87	1.86
CP, %	13.5	13.5	10.28	13.53	10.36
Ca, %	0.41	0.39	0.39	0.39	0.39
P, %	0.35	0.31	0.32	0.31	0.32
Available P, %	0.19	0.19	0.19	0.19	0.19
Stand. Dig. P, %	0.23	0.21	0.21	0.21	0.21
Ca:P	1.15	1.26	1.22	1.26	1.22
dEB <sup>3</sup> , mEQ/kg <sup>3</sup>	122	59	61	59	61

<sup>1</sup>Diets were fed from 252.8 to 301.7 lb BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for CP to use in formulation and total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.

<sup>2</sup>Axtra PHY (DuPont, Wilmington, DE) provided 150 phytase units (FTU) per lb of diet.

<sup>3</sup>Dietary electrolyte balance ( $435 \times \text{Na} + 256 \times \text{K} - 282 \times \text{Cl}$ ).<sup>1</sup>

<sup>1</sup> Austic, R. E., and C. C. Calvert. 1981. Nutritional interrelationships of electrolytes and amino acids. Fed. Proc. 40:63-67.

**Table 2. Effects of different AA:Lys ratios and CP on finishing pig growth performance and carcass characteristics<sup>1</sup>**

	Control diet <sup>2</sup>	AA:Lys ratio approach				SEM	Probability, <i>P</i> <		
		PIC		Modified			Ratio × CP	Control vs. PIC <sup>3</sup>	Control vs. modified <sup>3</sup>
CP, %:	13.5	13.5	10.3	13.5	10.3				
n	15	8	15	7	15	---	---	---	---
d 0 to 25									
ADG, lb	2.16	2.04	1.99	1.97	1.98	0.040	0.348	0.001	0.001
ADFI, lb	7.29	7.04	7.20	7.16	7.16	0.089	0.363	0.068	0.183
F/G	3.39	3.46	3.63	3.64	3.64	0.063	0.066	0.002	0.001
BW, lb									
d 0	252.6	252.7	252.8	253.1	252.7	2.45	0.780	0.868	0.761
d 25	304.6	302.2	301.0	300.4	300.3	1.35	0.627	0.017	0.001
Carcass characteristics									
HCW, lb	224.3	222.5	222.7	221.5	223.3	1.21	0.568	0.231	0.187
Yield, %	73.7	73.6	74.1	73.5	74.4	0.41	0.488	0.520	0.313
Backfat, in. <sup>4</sup>	0.65	0.63	0.65	0.64	0.65	0.024	0.650	0.469	0.760
Loin depth, in. <sup>4</sup>	2.94	2.91	2.93	2.94	2.94	0.024	0.506	0.223	0.784
Lean, % <sup>4</sup>	56.7	56.7	56.7	56.7	56.7	0.20	0.793	0.588	0.937

<sup>1</sup>A total of 1,682 pigs (PIC 327 × 1050, initially 252.7 lb BW) were used in a 25-d growth trial arranged in an unbalanced randomized complete block design with 25 pigs; however, a total of four pens (one from each treatment except treatment 13.5% CP and PIC ratio) were removed due to feeder flow ability issues.

<sup>2</sup>Diets were corn-soybean meal-based with 10% DDGS and 0.63% SID Lys. Treatments included 2 CP levels (10.3 vs. 13.5% using glycine and glutamic acid as nitrogen sources in a 2:1 ratio to increase CP) and 2 AA to Lys ratio approaches (PIC vs Modified) and a control with 13.5% CP using mainly soybean meal as the nitrogen source. The PIC and Modified ratio to Lys approaches were set at 55, 68, 17, 56, 65, 32 and 60, 68, 20, 55, 72, and 37% of Lys for Met+Cys, Thr, Trp, Ile, Val, and His, respectively.

<sup>3</sup>Contrast of control vs. PIC or Modified ratios independent of CP level.

<sup>4</sup>HCW was used as a covariate.

**Table 3. Main effects of different AA:Lys ratio approaches and CP on finishing pig growth performance and carcass characteristics<sup>1</sup>**

	AA:Lys ratio approach		SEM	Probability, <i>P</i> <	CP, %		SEM	Probability, <i>P</i> <
	PIC	Modified			13.5	10.3		
n	23	22	---	---	15	30	---	---
d 0 to 25								
ADG, lb	2.01	1.97	0.04	0.252	2.01	1.98	0.03	0.469
ADFI, lb	7.12	7.16	0.08	0.609	7.10	7.18	0.07	0.367
F/G	3.54	3.64	0.06	0.053	3.55	3.64	0.06	0.065
BW, lb								
d 0	252.8	252.9	2.4	0.877	252.9	252.8	2.4	0.872
d 25	301.6	300.3	1.3	0.275	301.3	300.6	1.2	0.554
Carcass characteristics								
HCW, lb	222.6	222.4	1.19	0.865	222.0	223.0	1.28	0.476
Yield, %	73.8	74.0	0.30	0.683	73.6	74.2	0.32	0.031
Backfat, in. <sup>3</sup>	0.64	0.64	0.230	0.677	0.64	0.65	0.237	0.377
Loin depth, in. <sup>3</sup>	2.92	2.94	0.024	0.338	2.92	2.93	0.024	0.608
Lean, % <sup>3</sup>	56.7	56.7	0.20	0.638	56.7	56.7	0.20	0.708

<sup>1</sup>A total of 1,682 pigs (PIC 327 × 1050, initially 252.7 lb BW) were used in a 25-d growth trial arranged in an unbalanced randomized complete block design with 25 pigs per pen; however, a total of five pens (one from each treatment except treatment 13.5% CP and PIC ratio) were removed due to feeder flow ability issues.

<sup>2</sup>Diets were corn-soybean meal based with 10% DDGS and 0.63% SID Lys. Treatments included 2 CP levels (10.3 vs. 13.5% using glycine and glutamic acid as nitrogen sources in a 2:1 ratio to increase CP) and 2 AA to Lys ratio approaches (PIC vs Modified) and a control with 13.5% CP using mainly soybean meal as the nitrogen source. The PIC and Modified ratio to Lys approaches were set at 55, 68, 17, 56, 65, 32 and 60, 68, 20, 55, 72, and 37% of Lys for Met+Cys, Thr, Trp, Ile, Val, and His, respectively.

<sup>3</sup>HCW was used as a covariate.

## Determination of the Optimum Levels of Dietary Crude Protein for Growth Performance and Carcass Characteristics of Finishing Pigs from 240 to 280 lb

*J.A. Soto, M.D. Tokach, S.S. Dritz,<sup>1</sup> J.C. Woodworth, J.M. DeRouchey, and R.D. Goodband*

### Summary

A total of 224 pigs (PIC 327 × 1050, initially 241.1 lb) were used in a 20-d trial to determine the optimum dietary CP concentration for growth performance and carcass characteristics of finishing pigs. Pens of 7 pigs were allotted by BW and randomly assigned to 1 of 4 dietary treatments with 7 or 8 replications per treatment. Dietary treatments included 4 levels of CP (10, 11, 12, and 13%) that were formed by reducing the amount of crystalline Lys in a corn-soybean meal diet. At d 20, pigs were transported to a packing plant for processing and carcass data collection. For overall growth performance (d 0 to 20), increasing CP increased (linear,  $P < 0.05$  and quadratic,  $P < 0.10$ ) ADG, ADFI, and HCW ADG with the greatest response for pigs fed the diet with 12% CP. Increasing diet CP also improved (linear,  $P < 0.05$ ) F/G, NE caloric efficiency, final BW, HCW, and HCW F/G. In conclusion, poorer performance of pigs fed diets under 12% CP was predominantly explained by feed intake but the mechanisms underlying regulation of feed consumption when feeding lower CP remains unclear.

Key words: amino acid, crude protein, finishing pigs

### Introduction

Multiple finishing pig studies have shown that a high-protein diet results in greater weight gain and higher carcass lean meat content (Adeola and Young, 1989<sup>2</sup>; Chiba et al., 2002<sup>3</sup>) of pigs fed a low-protein, amino acid fortified diet. Decreasing dietary protein may compromise pig growth and decrease carcass leanness (Tous et al., 2014<sup>4</sup>). One

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<sup>2</sup> Adeola, O., L.G. Young. 1989. Dietary protein-induced changes in porcine muscle respiration, protein synthesis and adipose tissue metabolism. *J. Anim. Sci.* 67:664-673.

<sup>3</sup> Chiba, L., D.L. Kuhlbers, L.T. Frobish, S.B. Jungst, E.J. Huff-Lonergan, S.M. Lonergan and K.A. Cummins. 2002. Effect of dietary restrictions on growth performance and carcass quality of pigs selected for lean growth efficiency. *Livest. Prod. Sci.* 74:93-102.

<sup>4</sup> Tous, N., R. Lizardo, B. Vila, M. Gispert, M. Font-i-Furnols and E. Estevez-Garcia. 2014. Effects of reducing dietary protein and lysine on growth performance, carcass characteristics, intramuscular fat, and fatty-acid profile of finishing barrows. *J. Anim. Sci.* 92:129-140.

possible explanation for these effects is that the low protein content restricted muscle growth, resulting in a surplus of energy being converted into intramuscular lipids (Tous et al., 2014). However, excessive CP intake has been shown to increase energy expenditure due to increased N excretion, as well as to impact organ size (Kerr et al., 2003<sup>5</sup>). Lenis and Jongbloed (1989)<sup>6</sup> reported that a 1% reduction in dietary CP content resulted in an 8.5% reduction in N excretion. Previous research has reported no performance effects of lowering CP in late finishing pigs when correct amino acid ratios are met; however, the reduction in CP was limited to 12% CP (Kerr et al., 2003), or different genetics and body weight range have been used (Tous et al., 2014). Recently, Soto et al. (2016)<sup>7</sup> studied the effects of feeding a 10 or 13% CP diet to finishing pigs and found significant performance reduction in pigs fed the diet with 10% CP. Overall, there is limited published research available to establish the optimal or minimum dietary CP level for late finishing pigs. Therefore, the objective of the present study is to determine the optimum levels of dietary crude protein for growth performance and carcass characteristics of finishing pigs from 240 to 280 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility was totally enclosed and environmentally regulated, containing 32 pens. Each pen was equipped with a dry single-sided feeder (Farmweld, Teutopolis, IL) and a 1-cup waterer. Pens were located over a completely slatted concrete floor with a 4-ft pit underneath for manure storage. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen.

A total of 224 pigs (PIC 327 × 1050, initially 241.1 lb) were used in a 20 d trial. There were 7 pigs per pen (4 barrows and 3 gilts) at a floor space of 8.95 ft<sup>2</sup> per pig. Pens were equipped with adjustable gates to allow space allowances per pig to be maintained if a pig died or was removed from a pen during the experiment. Pigs were allotted by BW and randomly assigned to 1 of 4 dietary treatments in a completely randomized block design. The dietary treatments included 4 CP concentrations (10, 11, 12, and 13%), with 7 replications for the treatment with 10% CP and 8 replications for the treatments with 11, 12, and 13% CP. Pigs were provided ad libitum access to water and feed in meal form. Prior to the trial, from 200 to 240 lb, these pigs were fed a corn-soybean meal-based diet with 14.2% CP, 0.72 standardized ileal digestible (SID) Lys and NE 1,150 Kcal/lb.

<sup>5</sup> Kerr, B. J., Yen, J., Nienaber and Easter. 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production in growing pigs. *J. Anim. Sci.* 81:1998-2007.

<sup>6</sup> Lenis, N. and A. Jongbloed. 1999. New technologies in low pollution swine diets: Diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission. *Asian-Aust. J. Anim. Sci.* 12(2):305-327.

<sup>7</sup> Soto, J.A., M.D. Tokach, S.S. Dritz, J.C. Woodworth, J.M. DeRouchey, and R.D. Goodband. 2016. Effects of dietary electrolyte balance and crude protein level on growth performance, carcass characteristics, and blood analytes of finishing pigs. Kansas State University Swine Industry Day, 2016. Kansas Agricultural Experiment Station Reports. 17-118-S. Vol: 2 Iss. 8.

To formulate the experimental diets, a 13% CP corn-soybean meal diet with 0.23% L-Lys HCl was formulated. Then L-lysine HCl was included at 0.52, 0.43, and 0.33% of the diet at the expense of soybean meal to reach the desired levels of 10, 11, and 12% CP, respectively (Table 1). Diets were isocaloric (NE kcal/lb 1,194) with all amino acids at or above minimum ratios relative to Lys.

Pigs were weighed on d 0, 7, 14, and 20 of the trial to determine ADG, ADFI, and F/G. At d 20, pigs were individually tattooed with a unique ID number to allow carcass measurements to be recorded on a pig basis. On d 20, final pen weights and individual weights were taken, and pigs were transported to a commercial packing plant (Farmland Crete, NE) for processing and determination of HCW.

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the experiment and stored at  $-4^{\circ}\text{F}$  until they were homogenized, subsampled, and submitted for analysis of DM, CP, Ca, P, ether extract, and ash (Cumberland Valley Analytical Services, Hagerstown, MD; Table 2).

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and initial BW as a blocking factor. Dietary treatments were the fixed effect and block served as the random effect in the analysis. Statistical significance was determined at  $P < 0.05$  and tendencies at  $P < 0.10$ .

## Results and Discussion

The analyzed DM, CP, Ca, P, ether extract, and ash contents of experimental diets (Tables 2) were reasonably consistent with formulated estimates.

For overall growth performance (d 0 to 20), increasing CP increased (linear,  $P < 0.05$  and quadratic,  $P < 0.10$ ) ADG and ADFI with the greatest response for pigs fed the diet with 12% CP with little improvement thereafter. In addition, increasing CP also improved (linear,  $P < 0.05$ ) F/G, caloric efficiency, and final BW.

For carcass characteristics, increasing CP increased (linear,  $P = 0.001$  and quadratic,  $P = 0.07$ ) HCW ADG with the greatest response for pigs fed the diet with 12% CP. Furthermore, HCW increased (linear,  $P = 0.040$ ) with increasing dietary CP without any influence on carcass yield. Also, HCW F/G, and HCW NE caloric efficiency improved (linear,  $P < 0.050$ ) with increasing CP.

In conclusion, the optimum dietary CP for ADG, ADFI, and HCW ADG were reached by pigs fed diets with 12% CP. Further improvement in HCW, F/G, caloric efficiency and HCW caloric efficiency were observed in pigs fed the diet with 13% CP. The F/G improvement in pigs fed the 13% CP diet may be due to underestimation of the concentration of NE in soybean meal by NRC (2012), as suggested by Sotak-Peper et al. (2015).<sup>8</sup> The poorer performance of pigs fed diets with less than 12% CP was predominantly explained by reduced feed intake, yet mechanisms underlying regulation of feed consumption when feeding lower CP remains unclear. In addition, it would

<sup>8</sup> Sotak-Peper, K.M., J.C. Gonzalez-Vega and H.H. Stein. 2015. Concentrations of digestible, metabolizable, and net energy in soybean meal produced in different areas of the United States and fed to pigs. J. Anim. Sci. 93:5694-5701.

possible to hypothesize that by reducing CP to low levels it may result in a deficiency of non-essential amino acids (Ball et al., 2013<sup>9</sup>), or other nutrients not provided by low CP diets. However, other research has suggested that late finishing pigs fed low CP diets supplemented with non-essential amino acids were not able to overcome the negative impacts on growth performance and carcass characteristics of the low CP (Rojo, 2011<sup>10</sup>). Further research is needed to understand the reasons that pigs fed diets with seemingly adequate levels of amino acids, but with less than 12% CP have reduced performance.

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<sup>9</sup> Ball M., E. Magowan, K. McCracken, V. Beattie, R. Bradford, F. Gordon, M. Robinson, S. Smyth and W. Henry. 2013. The effect of level of crude protein and available lysine on finishing pig performance, nitrogen balance and nutrient digestibility. *Asian-Aust. J. Anim. Sci.* 26(4):564-572.

<sup>10</sup> Rojo, A. 2011. Evaluation of the effects of branched chain amino acids and corn-distillers dried grains by-products on the growth performance, carcass and meat quality characteristics of pigs. Ph.D. diss., University of Illinois. Urbana-Champaign, IL.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Crude protein, %			
	10	11	12	13
Corn	93.09	89.87	86.63	83.38
Soybean meal (46.5% CP)	2.96	6.03	9.17	12.32
Choice white grease	0.55	1.00	1.45	1.90
Monocalcium P (21% P)	0.71	0.68	0.65	0.63
Limestone	0.97	0.98	0.96	0.92
Salt	0.35	0.35	0.35	0.35
L-Lys-HCl	0.52	0.43	0.33	0.23
DL-Met	0.10	0.07	0.04	0.02
L-Thr	0.19	0.15	0.11	0.06
L-Trp	0.06	0.05	0.03	0.01
L-Val	0.16	0.11	0.05	0.00
L-Ile	0.16	0.11	0.06	0.00
Trace mineral premix	0.10	0.10	0.10	0.10
Vitamin premix	0.08	0.08	0.08	0.08
Phytase <sup>2</sup>	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lys	0.66	0.66	0.66	0.66
Ile:Lys	65	65	65	65
Leu:Lys	132	143	154	165
Met:Lys	38	36	34	32
Met and Cys:Lys	62	62	62	62
Thr:Lys	66	66	66	66
Trp:Lys	19	19	19	19
Val:Lys	76	76	75	76
His:Lys	33	38	42	47
SID Lys: NE, g/Mcal	2.51	2.51	2.51	2.51
NE NRC, kcal/lb	1,194	1,194	1,194	1,194
CP, %	10.0	11.0	12.0	13.0
Ca, %	0.51	0.52	0.51	0.51
P, %	0.41	0.42	0.43	0.44
Available P, %	0.29	0.29	0.29	0.29
Standardized digestible P, %	0.31	0.32	0.32	0.32

<sup>1</sup>Diets were fed from d 0 to 20.

<sup>2</sup>Ronozyme Hiphos (GT) 2700 (DSM Nutritional Products, Inc, Parsippany, NJ). Provided 181.8 phytase units (FTU) per lb of diet with a release of 0.10% available P.

**Table 2. Chemical analysis of experimental diets (as-fed basis)<sup>1</sup>**

Item, %	Crude protein, %			
	10	11	12	13
DM	85.3	85.4	85.4	85.7
CP	9.0	10.9	11.9	13.1
Ca	0.72	0.62	0.60	0.61
P	0.46	0.56	0.48	0.50
Ether extract	3.7	5.4	5.1	5.3
Ash	4.0	4.2	4.0	4.1

<sup>1</sup>Multiple diet samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Cumberland Valley Analytical Service, Hagerstown, MD).

**Table 3. Effects of increasing dietary crude protein concentration on growth performance and carcass characteristics of finishing pigs from 240 to 280 lb<sup>1,2,3</sup>**

Item	Crude protein, %				SEM	Probability, <i>P</i> <	
	10	11	12	13		Linear	Quadratic
BW, lb							
d 20	276.8	278.9	281.4	280.9	1.40	0.022	0.341
BW CV, %							
d 0	9.2	8.3	8.4	8.5	1.03	0.650	0.600
d 20	7.8	6.8	8.4	7.8	0.84	0.670	0.770
d 0 to 20							
ADG, lb	1.69	1.89	2.01	1.99	0.068	0.001	0.080
ADFI, lb	5.69	5.99	6.26	6.09	0.127	0.014	0.060
F/G	3.35	3.18	3.11	3.06	0.086	0.020	0.452
NE Caloric efficiency <sup>4</sup>	4,033	3,804	3,736	3,662	102.8	0.010	0.430
Carcass characteristics							
HCW, lb	207.2	207.2	210.5	209.5	1.09	0.040	0.640
Carcass yield, %	74.8	74.3	74.8	74.6	0.24	0.780	0.510
Carcass CV, %	9.0	7.5	8.6	8.6	0.82	1.000	0.320
Carcass performance							
HCW ADG, lb	1.32	1.43	1.52	1.50	0.038	0.001	0.070
HCW F/G	4.31	4.20	4.15	4.07	0.087	0.050	0.880
NE Caloric efficiency	5,145	5,013	4,952	4,859	104.7	0.050	0.850

<sup>1</sup>A total of 224 pigs (PIC 1050 × 327; initially 241.1 lb) were used in a 20-d experiment with 7 pigs per pen.

<sup>2</sup>Allotment weight used as a covariate for growth performance, carcass characteristics, and carcass performance variables.

<sup>3</sup>Treatment with 10% CP had 7 replications and 8 replications for the treatments with 11, 12, and 13% CP.

<sup>4</sup>Caloric efficiency is expressed as kcal/lb of gain.

## Effects of Dietary Electrolyte Balance and Crude Protein Level on Growth Performance, Carcass Characteristics, and Blood Analytes of Finishing Pigs

*J.A. Soto, M.D. Tokach, S.S. Dritz,<sup>1</sup> J.C. Woodworth, J.M. DeRouchey, and R.D. Goodband*

### Summary

A total of 288 finishing pigs (PIC 327 × 1050, initially 243.5 lb) were used in a 20-d trial to determine if dietary electrolyte balance (dEB) in conjunction with low protein, amino acid fortified diets has any influence on growth performance. Pens of 8 pigs were allotted by BW and randomly assigned to 1 of 4 dietary treatments with 9 replications per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of CP (10 or 13%) and dEB (48 or 107 mEq/kg). At d 20, the pigs were transported to a packing plant for processing and carcass data collection. Pigs fed 13% CP diets had greater ( $P = 0.001$ ) ADG, heavier ( $P = 0.037$ ) final body weight, and improved ( $P < 0.001$ ) feed efficiency compared with pigs fed the 10% CP diets. A tendency for a CP × dEB interaction was observed for ADFI because intake numerically decreased when dEB was increased for pigs fed 10% CP, whereas intake increased as dEB was increased for pigs fed 13% CP diets. For carcass performance, pigs fed the diets with 13% CP had increased ( $P = 0.001$ ) HCW and HCW ADG and improved ( $P = 0.001$ ) HCW F/G compared with pigs fed the 10% CP diets. In conclusion, reduced performance observed in pigs fed the low crude protein diets with high supplemental crystalline AA was not influenced by dEB ranging from 48 to 107 mEq/kg. Dietary electrolyte balance in the range tested had no effects on growth performance, HCW, yield, or carcass performance during late finishing. Appropriate levels of dietary CP are critical to ensure optimal late finishing performance.

Key words: crude protein, electrolyte balance, late finishing

### Introduction

Economic and environmental pressures have obligated nutritionists to develop low protein, amino acid fortified diets that deliver performance equivalent to traditional formulations. However, in some studies, low protein diets lead to poorer performance

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in late finishing pigs.<sup>2</sup> By lowering crude protein, dietary electrolyte balance decreased proportionally. Dietary electrolyte balance (dEB) represents the net balance between fixed cations and anions (Na + K - Cl in mEq/kg of diet) and determines the net acid or alkaline load contributed by the diet. It is well known that dEB alters the acid-base status and subsequently may impact animal performance. Extensive research performed with dairy cattle, laying hens and lactating sows would indicate positive metabolic effects when dietary dEB is modified (DeRouchey et al., 2003<sup>3</sup>). In postweaned pigs, Guzman-Pino et al. (2015)<sup>4</sup> reported increased ADG and BW when dEB was increased from 16 to 133 mEq/kg. In finishing pigs, Patience et al. (1987)<sup>5</sup> reported increased ADFI when dEB was increased from 68 to 346 mEq/kg, although Wondra et al. (1995)<sup>6</sup> reported no changes in performance as dEB increased from 177 to 399 mEq/kg. Because the dEB is decreased when crystalline amino acids replace soybean meal in low crude protein diets, there is a need to establish whether dEB has any influence on finishing performance. Therefore, the objective of the present study is to determine the effects of dEB in diets with different levels of crude protein on growth performance, carcass characteristics, and blood analytes of pigs between 250 and 285 lb.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility was totally enclosed and environmentally regulated, containing 36 pens. Each pen was equipped with a dry single-sided feeder (Farmweld, Teutopolis, IL) and a 1-cup waterer. Pens were located over a completely slatted concrete floor with a 4-ft pit underneath for manure storage. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen.

A total of 288 pigs (PIC 327 × 1050, initially 243.5 lb) were used in a 20-d trial. There were 8 pigs per pen (4 barrows and 4 gilts) at a floor space of 7.83 ft<sup>2</sup> per pig and 9 replications per treatment. Pens were equipped with adjustable gates to allow space allowances per pig to be maintained if a pig died or was removed from a pen during the experiment. Pigs were allotted by BW and randomly assigned to 1 of 4 dietary treatments in a completely randomized block design. Treatments were arranged in a 2 × 2 factorial with main effects of CP (10 or 13%) and dEB (48 or 107 mEq/kg).

<sup>2</sup> Vonderohe, C.E., K.M. Mills, M. D., Asmus, E. R. Otto-Tice, C.V. Maxwell, B.T., Richert, and J.S. Radcliffe. 2016. Comparison of the effects of reduced CP, amino acid supplemented diets on growth performance in swine *J. Anim. Sci.* 94:16 (Abstract).

<sup>3</sup> DeRouchey, J.M., J.D. Hancock, R.H. Hines, K.R. Cummings, D.J. Lee, C.A. Maloney, D.W. Dean, J.S. Park, and H. Cao. 2003. Effects of dietary electrolyte balance on the chemistry of blood and urine in lactating sows and sow litter performance. *J. Anim. Sci.* 81:3067-3074.

<sup>4</sup> Guzmán-Pino, S.A., D. Sola-Oriol, R. Davin, E. G. Manzanilla, and J. F. Pérez. 2015. Influence of dietary electrolyte balance on feed preference and growth performance of post weaned piglets. *J. Anim. Sci.* 2015. 93:2840-2848.

<sup>5</sup> Patience, J.F., R.E. Austic, and R.D. Boyd. 1987. Effect of dietary electrolyte balance on growth performance and acid-base status in swine *J. Anim. Sci.* 64: 457-466.

<sup>6</sup> Wondra, K.J., J.D. Hancock, K.C. Behnke, and R.H. Hines. 1995. Effect of dietary buffers on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *J. Anim. Sci.* 73: 414-420.

To formulate the experimental diets, a 13% CP corn-soybean meal diet was formulated to include a moderate level (0.23%) of L-lysine HCl with all other amino acids at or above minimum ratios relative to lysine. Dietary electrolyte balance in this diet was 107 mEq/kg. Then the CP was decreased to 10% by increasing the inclusion of crystalline amino acids resulting in a diet with a dEB of 48 mEq/kg. Again, all amino acids were at or above minimum ratios relative to lysine. To complete the factorial, calcium chloride was added (0.43%) to the 13% CP diet to lower dEB from 107 to 48 mEq/kg and sodium bicarbonate was added (0.51%) to the 10% CP diet to increase dEB from 48 to 107 mEq/kg (Table 1).

Pigs were weighed on d 0, 7, 14, and 20 of the trial to determine ADG, ADFI, and F/G. At d 19 of the trial, blood samples of 72 pigs (2 gilts per pen, 18 gilts per treatment) were collected and submitted to the Kansas State Veterinary Diagnostic Laboratory to determine blood urea nitrogen (mg/dl), Ca (mg/dl), Na (mmol/L), K (mmol/L), and Cl (mmol/L). Blood was collected from the jugular vein. Bleeding was started at 0700 and all pigs were bled within 60 min. Feed was not withheld before the bleeding period. For all blood analytes, the Roche Cobas c501 analyzer was used (Roche Diagnostics Corporation, Indianapolis, IN). The blood urea nitrogen (BUN) and Ca concentrations were determined photometrically and Na, K, and Cl electrical potential were measured by ion selective electrodes. At d 20, the pigs were individually tattooed with a unique ID number to allow carcass measurements to be recorded on a pig basis. On d 20, final pen weights and individual weights were taken, and pigs were transported to a commercial packing plant (Farmland Crete, NE) for processing and determination of HCW.

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the experiment and stored at  $-4^{\circ}\text{F}$  until they were homogenized, subsampled, and submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, Ca, P, crude fat, and ash (Table 2).

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and initial BW as a blocking factor. Dietary treatments were the fixed effect and block served as the random effect in the analysis. Statistical significance was determined at  $P < 0.05$  and tendencies at  $P < 0.10$ .

## Results and Discussion

The analyzed DM, CP, Ca, P, fat, ash, and dEB contents of experimental diets were reasonably consistent with formulated estimates (Table 2).

For overall growth performance (d 0 to 20), pigs fed diets with 13% CP had increased ( $P = 0.001$ ) ADG compared with pigs fed diets with 10% CP which resulted in a heavier ( $P = 0.037$ ) final BW. Pigs fed the diets with 13% CP had improved ( $P < 0.001$ ) F/G compared with pigs fed the 10% CP diets. A tendency for a CP  $\times$  dEB interaction was observed for ADFI ( $P = 0.081$ ) because intake was numerically reduced when dEB increased for the pigs fed 10% CP, whereas intake increased as dEB was increased for the pigs fed 13% CP. The grams of SID Lys intake per kilogram of gain were lower ( $P < 0.001$ ) for pigs fed the diets with 13% CP in comparison with pigs fed the diets fed 10% CP. For both levels of CP, the grams of SID Lys intake were on the higher end

of the NRC (2012) requirements estimates of 14.6 to 24.7 g per kilogram of gain for finishing pigs.

For carcass performance, pigs fed the diets with 13% CP had increased ( $P = 0.001$ ) HCW ADG compared with pigs fed the 10% CP diets. Pigs fed the diets with 13% CP had improved ( $P = 0.001$ ) HCW F/G compared with pigs fed the 10% CP diets. No main effects for either CP or dEB were observed for HCW and carcass yield.

For blood analytes, a CP  $\times$  dEB interaction ( $P = 0.029$ ) was observed for BUN. The interaction was similar to the interaction for ADFI with BUN numerically decreasing for pigs fed 10% CP as dEB was increased, while BUN increased as dEB increased for pigs fed 13% CP. Pigs fed the diets with 10% CP had increased ( $P = 0.002$ ) Na compared with pigs fed diets with 13% CP. Pigs fed the diets with 48 mEq/kg of dEB had increased ( $P = 0.048$ ) Cl compared with pigs fed diets with 107 mEq/kg of dEB.

In conclusion, reduced performance observed in pigs fed the low CP diets with higher supplemental crystalline AA was not influenced by dEB ranging from 48 to 107 mEq/kg. Dietary electrolyte balance had no effects on growth performance, HCW, yield, or carcass characteristics during late finishing. The reason for the low performance of pigs fed diets containing 10% CP is unknown, but does not appear to be related to dEB. Appropriate levels of dietary CP are critical to ensure optimal late finishing performance.

**Table 1. Diet composition per treatments (as-fed basis)<sup>1</sup>**

Ingredient, %	Crude protein, %: dEB, mEq/kg:	10		13	
		48	107	48	107
Corn		92.64	91.82	82.77	83.00
Soybean meal, (46.5% CP)		3.29	3.35	12.51	12.49
Choice white grease		0.55	0.80	2.00	1.90
Monocalcium P, (21% P)		0.50	0.50	0.45	0.45
Limestone		1.35	1.35	0.98	1.30
Salt		0.35	0.35	0.35	0.35
L-Lys-HCl		0.51	0.51	0.23	0.23
DL-Met		0.08	0.08	0.03	0.03
L-Thr		0.19	0.19	0.06	0.06
L-Trp		0.06	0.06	0.01	0.01
L-Val		0.15	0.15	0.00	0.00
L-Ile		0.15	0.15	0.00	0.00
Trace mineral premix		0.10	0.10	0.10	0.10
Vitamin premix		0.08	0.08	0.08	0.08
Phytase <sup>2</sup>		0.02	0.02	0.02	0.02
Calcium chloride		0.00	0.00	0.43	0.00
Sodium bicarbonate		0.00	0.51	0.00	0.00
Total		100	100	100	100
Calculated analysis					
Standardized ileal digestible amino acids, %					
Lys		0.66	0.66	0.66	0.66
Ile:Lys		64	64	65	65
Leu:Lys		133	132	165	165
Met:Lys		36	36	34	34
Met and Cys:Lys		60	60	64	64
Thr:Lys		66	67	66	66
Trp:Lys		19	19	19	19
Val:Lys		75	75	76	76
SID Lys: NE, g/Mcal		2.51	2.51	2.51	2.51
NE, kcal/lb		1,191	1,191	1,191	1,191
CP, %		10.1	10.1	13.1	13.1
Ca, %		0.61	0.61	0.61	0.61
P, %		0.37	0.37	0.40	0.40
Available P, %		0.25	0.25	0.25	0.25
Standardized digestible P, %		0.28	0.28	0.29	0.29

<sup>1</sup>Diets were fed from d 0 to 20.

<sup>2</sup>Ronozyme Hiphos (GT) 2700 (DSM Nutritional Products, Inc, Parsippany, NJ). Provided 181.8 phytase units (FYT) per pound of diet and an estimated release of 0.10% available P.

**Table 2. Chemical analysis of experimental diets (as-fed basis)<sup>1</sup>**

Item	Crude protein, %:	10		13	
	dEB, mEq/kg:	48	107	48	107
DM, %		87.7	86.9	87.5	87.5
CP, %		9.8	9.2	11.9	12.6
Ca, %		0.60	0.75	0.63	0.63
P, %		0.42	0.42	0.41	0.42
Na, %		0.12	0.33	0.17	0.14
Cl, %		0.36	0.42	0.56	0.30
K, %		0.44	0.41	0.55	0.54
Ether extract, %		4.1	3.9	4.8	4.5
Ash, %		2.41	3.07	3.07	2.97
Analyzed dEB, mEq/kg <sup>2</sup>		63	114	57	130

<sup>1</sup>Multiple diet samples were collected from each diet throughout the study, homogenized, then subsampled for analysis at Ward Laboratories, Inc. (Kearney, NE).

<sup>2</sup>dEB, mEq/kg = (Na% × 434.98) + (K% × 255.74) - (Cl% × 282.06).

**Table 3. Effects of dietary electrolyte balance and crude protein level on growth performance, carcass characteristics, and blood analytes of finishing pigs<sup>1,2</sup>**

Crude protein, %: dEB, mEq/Kg:	10		13		SEM	Probability, <i>P</i> <		
	48	107	48	107		CP × dEB	CP	dEB
BW, lb								
d 0	243.6	243.5	243.5	243.5	1.26	0.178	0.699	0.247
d 20	274.1	273.3	275.5	277.5	1.57	0.291	0.037	0.657
BW CV, %								
d 0	8.25	8.75	8.57	8.84	0.650	0.858	0.758	0.554
d 20	8.13	7.81	8.41	8.10	0.647	0.997	0.657	0.628
D 0 to 20								
ADG, lb	1.58	1.56	1.69	1.78	0.046	0.236	0.001	0.442
ADFI, lb	6.24	6.12	6.06	6.38	0.138	0.083	0.730	0.451
F/G	3.96	3.93	3.60	3.57	0.087	0.948	<0.001	0.734
SID Lys, g/kg gain	26.1	25.9	23.7	23.7	0.57	0.967	<0.001	0.742
Carcass characteristics								
HCW, lb	209.9	209.7	210.1	212.1	1.45	0.420	0.329	0.511
Carcass yield, %	74.09	74.28	73.95	73.96	0.224	0.690	0.304	0.651
HCW CV, %	8.73	8.33	9.80	8.30	0.712	0.445	0.465	0.191
Carcass performance								
HCW ADG, lb	1.17	1.16	1.25	1.32	0.034	0.263	0.002	0.386
HCW F/G	5.34	5.29	4.86	4.84	0.119	0.898	<0.001	0.709
Blood analytes								
Na mmol/L	147.1	147.4	145.8	146.4	0.37	0.726	0.002	0.123
K mmol/L	5.2	5.1	5.0	5.0	0.11	0.754	0.190	0.613
Cl mmol/L	102.0	101.4	101.7	100.8	0.36	0.598	0.205	0.048
Ca mg/dL	11.3	11.4	11.2	11.3	0.09	0.883	0.212	0.174
BUN mg/dL	5.7	4.1	9.3	10.1	0.56	0.029	<0.001	0.488

<sup>1</sup>A total of 288 pigs (PIC 1050 × 327; initially 243.5 lb) were used in a 20-d experiment with 8 pigs per pen and 9 pens per treatment.

<sup>2</sup>Sodium bicarbonate was added to the diet with 10% CP to increase dEB to 107 mEq/kg. Calcium chloride was added to the diet with 13% CP to lower dEB to 48 mEq/kg.

## Evaluation of Dietary Phytochemicals on Growth Performance, Carcass Characteristics, and Economics of Grow-finish Pigs Housed Under Commercial Conditions<sup>1</sup>

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### Summary

A total of 1,260 pigs (PIC 327 × 1050, initially 48.7 lb) were used in a 125-d trial to determine the effect of two dietary essential oil mixtures on the growth performance, carcass characteristics, and economics of finishing pigs. Pigs were allotted by BW and randomly assigned to 1 of 5 dietary treatments. Pigs were fed six dietary phases. Treatment 1 was the control with no feed additives and 12% of CP in the Phase 6 diet. Treatment 2 was the same formulation as treatment 1 but contained an essential oil mixture 1 (EOM 1) containing caraway, garlic, thyme, and cinnamon fed all phases. Treatment 3 was the same formulation as treatment 1 with EOM 1 fed from Phases 3 to 6 and essential oil mixture 2 (EOM 2) containing oregano, citrus, and anise fed all phases (EOM 1+2). Treatment 4 contained EOM 1 fed in all 6 phases with 16% CP in Phase 6. Treatment 5 contained ractopamine HCl (9 g/ton) with 16% CP in the Phase 6 diet. Overall (d 0 to 125), pigs fed diets with EOM 1+2 had increased ( $P = 0.003$ ) ADFI compared with pigs fed the control treatment. Pigs fed the diet with EOM 1 and 16% CP had increased ( $P = 0.032$ ) ADFI in comparison with the pigs fed ractopamine HCl treatment. Pigs fed the ractopamine HCl treatment had improved ( $P = 0.028$ ) F/G compared with pigs fed the treatment with the EOM 1 and 16% CP and the control treatment. For carcass traits, pigs fed the treatment with EOM 1+2 and had increased ( $P = 0.007$ ) HCW compared with pigs fed EOM 1 and 12% CP and the control treatment ( $P = 0.002$ ). Pigs fed the treatment with ractopamine HCl also had heavier ( $P = 0.001$ ) HCW compared with the control treatment. Pigs fed diets with EOM 1+2 had increased ( $P = 0.001$ ) carcass ADG, compared with pigs fed the control treatment and the treatment with EOM 1 and 12% CP ( $P = 0.019$ ). Pigs fed the treatment with ractopamine HCl also had improved ( $P = 0.001$ ) carcass ADG compared with pigs fed

<sup>1</sup> Appreciation is expressed to Biomin America Inc. (San Antonio, TX) for providing the phytochemical products and financial support, New Horizons Farms (Pipestone, MN) for providing animals and research facilities and to Marty Heintz for technical assistance.

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the control treatment. Pigs fed diets with EOM 1+2 had increased ( $P = 0.021$ ) carcass yield compared with pigs fed the treatment with EOM 1 and 12% CP. Carcass yield was improved ( $P = 0.036$ ) for the treatment with ractopamine HCl in comparison with the control treatment. Economically, feed cost per pound of gain was lower ( $P < 0.001$ ) for pigs fed the control treatment compared to the treatment with EOM 1+2 and pigs fed with the ractopamine HCl treatment. Pigs fed diets with EOM 1+2 or ractopamine HCl treatment had increased ( $P = 0.001$ ) gain value compared with pigs fed the control treatment. Pigs fed the ractopamine HCl treatment had increased income over feed cost in comparison with the treatments containing EOM 1 with 16% CP. In conclusion, the addition of EOM 1+2 improved ADFI, HCW, carcass ADG, and gain value in comparison with the control treatment. However, the increase in gain was not sufficient to overcome the increase in feed cost. The gain value improvement for the regimen with ractopamine HCl compensated for the extra feed cost resulting in a higher income over feed cost compared with the treatment with EOM 1 and 16% CP.

Key words: essential oils, feed additives, phytochemicals

## Introduction

Phytochemical feed additives are compounds derived from plant extracts incorporated into animal feed with the goal of improving animal health and performance. While the exact mode of action and physiological effects are not fully understood, most are associated with antimicrobial benefits, increased antioxidant activity, and improved gut function (Jacela et al., 2010<sup>3</sup>). Additionally, phytochemicals potentially can increase diet palatability, which could lead to higher growth rates (Windisch et al., 2007<sup>4</sup>; Karaskova et al., 2015<sup>5</sup>).

Within the phytochemical classification, the active substances found in the products may vary widely depending upon the plant species, plant part used, harvesting season, crop density, and geographical origin (Windisch et al., 2007). Currently, phytochemical additives have been predominantly provided through essential oils. Essential oils are complex mixtures of volatile and lipophilic compounds. Due to their lipophilicity, they are associated with good intestinal absorption. The intake of phytochemicals can stimulate the secretion of digestive enzymes and increase gastric and intestinal motility (Yang et al., 2012<sup>6</sup>). Research with phytochemicals in swine diets has yielded inconsistent results with more research needed to determine the correct blend or timing of use as well as to identify the greatest opportunities to yield economic benefits (Yang et al., 2012; Thacker, 2014<sup>7</sup>). Therefore, the objective of this study was to determine the effect of

<sup>3</sup> J. Jacela, J. DeRouchey, M. Tokach, R. Goodband, J. Nelssen, D. Renter and S. Dritz. 2010. Feed additives for swine: Fact sheets – prebiotics and probiotics, and phytochemicals. J. Swine Health Prod. 2010; 18(3): 132-136.

<sup>4</sup> W. Windisch, K. Schedle, C. Plitzner and A. Kroismayr. 2007. Use of phytochemical products as feed additives for swine and poultry. J. Anim. Sci. 86:140-148.

<sup>5</sup> K. Karaskova, P. Suchy and E. Strakova. 2015. Current use of phytochemical feed additives in animal nutrition: a review. Czech J. Anim. Sci. 60:521-530.

<sup>6</sup> L. Yan, q. Meng, and I. Kim. 2012. Effect of an herb extract mixture on growth performance, nutrient, digestibility, blood characteristics, and fecal microbial shedding in weanling pigs. Livest. Sci. 145:189-195.

<sup>7</sup> Thacker, P. 2013. Alternatives to antibiotics as growth promoters for use in swine productions: a review. J. Anim. Sci. Biotechnol. 4(1):1-12.

dietary phytochemicals on the growth performance, carcass characteristics, and economics of grow-finish pigs housed under commercial conditions.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research-finishing site in southwest Minnesota from August to December. The barn was naturally ventilated and double-curtain-sided. Each pen was equipped with a 5-hole stainless steel feeder and bowl waterer for ad libitum access to feed and water. Feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 1,260 mixed gender pigs (PIC 1050 × 327, initially 47.8 lb) were used in a 125-d trial. There were 28 pigs per pen (6.78 square ft<sup>2</sup> per pig), and 9 replications per treatment with a similar number of barrows and gilts in each pen. Pigs were allotted based on initial body weight to pens assigned to 1 of 5 treatments in a completely randomized block design.

Pigs were fed a conventional nutritional program with a total of six dietary phases (Tables 1 and 2). Treatment 1 was the control with no feed additives and 12% CP in Phase 6 diet. Treatment 2 was the same formulation as treatment 1 but contained an essential oil mixture 1 (EOM 1) of caraway, garlic, thyme, and cinnamon fed in all phases with an inclusion rate of 0.015%. Treatment 3 was the same diet formulation as treatment 1, but with EOM 1 fed from Phase 3 to 6 and essential oil mixture 2 (EOM 2) of oregano, citrus, and anise fed in all phases with an inclusion rate of 0.015% and 0.0125%, respectively (EOM 1+2). Treatment 4 contained EOM 1 fed in all 6 phases with 16% CP in the Phase 6 diet, with an inclusion rate of 0.015%. Treatment 5 contained 9 g/ton of ractopamine HCL (Paylean; Elanco Animal Health, Greenfield, IN) with 16% CP in the Phase 6 diet.

Pigs were weighed on d 0, 13, 28, 47, 70, 90, 106, and 125 of the trial to determine ADG, ADFI, and F/G. On d 106, the 3 heaviest pigs in each pen were weighed and sold according to standard farm procedures. Prior to marketing, the remaining pigs were individually tattooed with a pen ID number to allow for carcass measurements to be recorded on a pen basis. On d 125, final pen weights were taken, and pigs were transported to a USDA-inspected packing plant (JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included HCW, loin depth, backfat, and percentage lean. Percentage lean was calculated from plant proprietary equation. Carcass yield was then calculated by dividing the individual HCW at the plant by the pig's pen average final live weight at the farm.

An economic analysis was completed to determine the financial impact of the dietary treatments. Income over feed cost was calculated assuming that other costs, such as utility and labor, are equal across treatments and the only variables are carcass ADG and feed usage for the experimental period. Corn was valued at \$137/ton, soybean meal at \$288/ton, dried distillers grains with solubles at \$130/ton, L-Lys HCl at \$0.70/lb, EOM 1 at \$10.91/lb, EOM 2 at \$21.81/lb, and ractopamine HCl at \$32.00/lb. The total feed cost per pig was calculated by multiplying the ADFI by the feed cost per pound

and the number of days in each respective period, then taking the sum of those values for each period. Cost per pound of gain was calculated by dividing the total feed cost per pig by the total pounds gained overall. Gain value per pig was calculated by multiplying carcass gain by an assumed carcass value of \$70.00 per cwt. To calculate income over feed cost (IOFC), total feed cost was subtracted from gain value per pig.

Diet samples from each dietary phase were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of each dietary phase and stored at  $-4^{\circ}\text{F}$  until they were homogenized, subsampled and submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, Ca, P, crude fat, and ash (Tables 3, 4, and 5).

Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and initial BW as a blocking factor. Dietary treatments were the fixed effect and block served as the random effect in the analysis. HCW was used as a covariate for analyses of backfat thickness, loin depth, and percentage lean. When a significant difference was found between treatments, differences were determined using the PDIF statement in SAS. Statistical significance was determined at  $P < 0.05$ .

## Results and Discussion

The analyzed DM, CP, Ca, P, fat, and ash content of experimental diets (Tables 3, 4, and 5) were consistent with formulated estimates with the exception of the EOM1 diets in Phases 1 and 2, which analyzed lower in CP than expected.

For overall growth performance (d 0 to 125), pigs fed diets with EOM 1+2 had increased ( $P = 0.003$ ) ADFI compared with pigs fed the control treatment. The higher ADFI promoted a numerical ( $P > 0.050$ ) improvement in ADG. Pigs fed the treatment with EOM 1 and 16% CP had increased ( $P = 0.032$ ) ADFI in comparison with the ractopamine HCl treatment. Pigs fed the ractopamine HCl treatment had improved ( $P = 0.028$ ) F/G compared with pigs fed the treatment with the EOM 1 and 16% CP.

For carcass traits, pigs fed the treatment with EOM 1+2 had increased ( $P = 0.007$ ) HCW compared with the treatment with EOM 1 and 12% CP and the control treatment ( $P = 0.002$ ). Pigs fed the treatment with ractopamine HCl had improved ( $P = 0.001$ ) HCW compared with the control treatment. Pigs fed diets with EOM 1+2 had increased ( $P = 0.001$ ) carcass ADG compared with pigs fed the control treatment and the treatment with EOM 1 and 12% CP ( $P=0.019$ ). Pigs fed the treatment with ractopamine HCl had improved ( $P = 0.001$ ) carcass ADG compared with the control treatment. Pigs fed the treatment with ractopamine HCl had improved ( $P=0.001$ ) carcass F/G in comparison with the treatment with EOM 1 and 16% CP and the control treatment ( $P < 0.001$ ) with no differences among the other treatments. Pigs fed diets with EOM 1+2 had increased ( $P = 0.021$ ) carcass yield compared with pigs fed the treatment with EOM 1 and 12% CP. Carcass yield also was improved ( $P = 0.036$ ) for the treatment with ractopamine HCl in comparison with the control treatment. Pigs fed the ractopamine HCl had reduced ( $P = 0.001$ ) backfat in comparison with pigs fed the treatment with EOM 1 and 16% CP, or the control treatment ( $P < 0.001$ ). Pigs fed the control treatment or treatments with EOM 1 or EOM 1+2 with 12% CP had similar backfat. Pigs fed the treatment with ractopamine HCl had increased ( $P = 0.002$ )

lean percentage in comparison with pigs fed the treatment with EOM 1 and 16% CP, or the control treatment ( $P = 0.002$ ).

For economics, total feed cost per pig was lower ( $P = 0.006$ ) for pigs fed the control treatment compared to the treatment with EOM 1 and the treatment with EOM 1+2, both with 12% CP ( $P < 0.001$ ). Pigs fed the treatment with EOM 1+2 had higher ( $P = 0.001$ ) feed cost per pig in comparison with the treatment with EOM 1 and 12% CP. Pigs fed the treatment with ractopamine HCl treatment had higher ( $P < 0.001$ ) feed cost compared with the control treatment. Feed cost per pound of gain was lower ( $P < 0.001$ ) for pigs fed the control treatment compared to the treatment with EOM 1+2. Pigs fed with the ractopamine HCl treatment had higher ( $P = 0.023$ ) feed cost per pound of gain compared with the control treatment. Pigs fed diets with EOM 1+2 had increased ( $P = 0.001$ ) gain value compared with pigs fed the control treatment. Pigs fed the treatment with ractopamine HCl had increased ( $P = 0.001$ ) gain value in comparison with the control treatment.

In summary, the addition of the combination of EOM 1+2 to the diets improved ADFI, HCW, and carcass ADG in comparison with the control treatment. The inclusion of this treatment improved gain value, however, the increase in gain was not sufficient to overcome the increase in feed cost. Thus, income over feed cost was similar to the control treatment. Regardless of the lower ADFI with the ractopamine HCl treatment in comparison with pigs fed EOM 1 and 16% CP, the treatment with ractopamine HCl had improved F/G, carcass F/G, lean percentage, and reduced backfat. The gain value improvement for the regimen with ractopamine HCl compensated for the extra feed cost, resulting in a higher income over feed cost compared with the treatment with EOM 1 and 16% CP. Similar positive effects in growth and carcass characteristics were observed with the ractopamine HCl treatment compared with the control treatment; however, income over feed cost was similar for both treatments. The addition of combined essential oils provided with positive effects for growth and carcass characteristics for grow-finish pigs. However, the magnitude of the benefits value did not economically justify their inclusion into the diets. As expected, the ractopamine HCl had positive effects on growth and carcass characteristics, nonetheless it provided only numerical differences in income over feed cost in comparison with the control treatment.

**Table 1. Diet composition from Phase 1 to 5 (as-fed basis)<sup>1,2</sup>**

Item	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Ingredient, %					
Corn	59.36	65.13	70.50	74.05	76.42
Soybean meal, (46.5% CP)	23.13	17.48	12.24	8.85	6.44
DDGS <sup>3</sup>	15.00	15.00	15.00	15.00	15.00
Limestone	1.10	1.10	1.05	1.00	1.00
Monocalcium P, (21% P)	0.25	0.15	0.10	0.05	0.05
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys-HCl	0.41	0.41	0.41	0.42	0.42
DL-Met	0.09	0.06	0.04	0.02	0.02
L-Thr	0.10	0.10	0.09	0.09	0.09
L-Trp	0.03	0.04	0.04	0.04	0.04
Phytase <sup>4</sup>	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.10	0.10	0.10	0.06	0.10
Vitamin premix	0.08	0.08	0.08	0.06	0.06
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Standardized ileal digestible amino acids, %					
Lys	1.12	0.98	0.85	0.77	0.71
Ile:Lys	61	60	58	57	56
Leu:Lys	139	145	152	157	162
Met:Lys	32	32	31	30	30
Met and Cys:Lys	56	56	56	56	57
Thr:Lys	62	62	62	62	63
Trp:Lys	19	19	18	19	18
Val:Lys	67	67	67	67	67
SID lysine: ME, g/Mcal	3.38	2.95	2.55	2.31	2.13
ME, kcal/lb	1,503	1,507	1,511	1,515	1,515
CP, %	19.7	17.4	15.3	14.0	13.0
Ca, %	0.57	0.53	0.49	0.45	0.44
P, %	0.46	0.42	0.38	0.36	0.35
Available P, %	0.30	0.28	0.26	0.24	0.24
Standardized digestible P, %	0.34	0.31	0.29	0.27	0.26

<sup>1</sup>Phases 1, 2, 3, 4, and 5 diets were fed from d 0 to 13, d 13 to 47, d 47 to 70, 70 to 90, and d 90 to 106, respectively.

<sup>2</sup>EOM 1 was included at 0.015% in all 6 phases only for treatments 2 and 4. EOM 1 was included at 0.015% from Phase 3 to 6 and EOM 2 was included at 0.0125% from Phase 1 to 6 only for treatment 3.

<sup>3</sup>Dried distillers grains with solubles.

<sup>4</sup>Optiphos 2000 (Enzyvia LLC, Sheridan, IN) provided 136.5 FTU per pound of diet.

**Table 2. Phase 6 diet composition (as-fed basis)<sup>1,2</sup>**

Item	Control	EOM 1	EOM 1+2	EOM 1	Ractopamine HCl
Ingredient, %					
Corn	85.50	85.48	85.47	76.13	76.10
Soybean meal, (46.5% CP)	12.38	12.38	12.38	21.66	21.66
Limestone	1.00	1.00	1.00	1.00	1.00
Monocalcium (21% P)	0.25	0.25	0.25	0.20	0.20
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys-HCl	0.23	0.23	0.23	0.25	0.25
DL-Met	0.03	0.03	0.03	0.09	0.09
L-Thr	0.08	0.08	0.08	0.12	0.12
L-Trp	0.02	0.02	0.02	0.02	0.02
Ractopamine HCl <sup>3</sup>	0.00	0.00	0.00	0.00	0.05
Phytase <sup>4</sup>	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.06	0.06	0.06	0.06	0.06
EOM 1	---	0.015	0.015	0.015	---
EOM 2	---	---	0.0125	---	---
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Standardized ileal digestible amino acids, %					
Lysine	0.65	0.65	0.65	0.90	0.90
Ile:Lys	63	63	63	63	63
Leu:Lys	155	155	155	137	137
Met:Lys	32	32	32	35	35
Met and Cys:Lys	60	60	60	60	60
Thr:Lys	67	67	67	67	67
Trp:Lys	19	19	19	19	19
Val:Lys	72	72	72	69	69
SID lys:ME, g/Mcal	1.95	1.95	1.96	2.71	2.71
ME, kcal/lb	1,509	1,508	1,508	1,506	1,505
CP, %	12.2	12.2	12.2	16.0	16.0
Ca, %	0.49	0.49	0.49	0.51	0.51
P, %	0.36	0.36	0.36	0.39	0.39
Available P, %	0.23	0.23	0.23	0.23	0.23
Standard digestible P, %	0.27	0.27	0.27	0.29	0.29

<sup>1</sup>Phase 6 diets were fed from d 106 to 125.

<sup>2</sup>EOM 1 was included at 0.015% in all 6 phases only for treatments 2 and 4. EOM 1 was included at 0.015% from Phase 3 to 6 and EOM 2 was included at 0.0125% from Phases 1 to 6 for treatment 3 only.

<sup>3</sup>Paylean (Elanco Animal Health, Greenfield, IN).

<sup>4</sup>Optiphos 2000 (Enzyvia LLC, Sheridan, IN) provided 136.5 FTU per pound of diet.

**Table 3. Chemical analysis of experimental diets (as-fed basis)<sup>1,2</sup>**

Feed additive:	Phase 1			Phase 2		
	Control <sup>3</sup>	EOM 1 <sup>4</sup>	EOM 1+2 <sup>5</sup>	Control	EOM 1	EOM 1+2
Item, %						
DM	89.6	89.2	89.6	89.4	88.9	88.6
CP	21.2	17.8	20.1	18.6	16.8	19.1
Ca	0.66	0.61	0.70	0.66	0.61	0.60
P	0.47	0.46	0.47	0.46	0.43	0.44
Ether extract	2.9	3.0	3.0	3.4	3.0	2.9
Ash	4.0	3.9	4.4	3.7	3.8	3.9

<sup>1</sup> Multiple diet samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Ward Laboratories, Inc. Kearney, NE).

<sup>2</sup> Phases 1 and 2 were fed from d 0 to 13 and d 13 to 47, respectively.

<sup>3</sup> Control treatment (T1) had the same formulation to the ractopamine HCL treatment (T5) until Phase 5.

<sup>4</sup> EOM 1 was included at 0.02% in all 6 phases for treatments 2 and 4.

<sup>5</sup> EOM 1 was included at 0.02% for Phase 3 to 6 and EOM 2 was included at 0.01% for Phase 1 to 6 for treatment 3.

**Table 4. Chemical analysis of experimental diets (as-fed basis)<sup>1,2</sup>**

Feed additive:	Phase 3			Phase 4		
	Control <sup>3</sup>	EOM 1 <sup>4</sup>	EOM 1+2 <sup>5</sup>	Control	EOM 1	EOM 1+2
Item, %						
DM	88.8	88.8	89.1	88.4	89.1	88.6
CP	14.7	15.7	15.7	14.1	14.6	15.0
Ca	0.52	0.51	0.54	0.60	0.45	0.49
P	0.38	0.41	0.38	0.40	0.38	0.42
Ether extract	3.1	3.5	3.4	3.3	4.0	4.0
Ash	3.3	3.4	3.3	3.2	3.2	3.2

<sup>1</sup> Multiple diet samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Ward Laboratories, Inc. Kearney, NE).

<sup>2</sup> Phase 3 and 4 were fed from d 47 to 70 and d 70 to 90, respectively.

<sup>3</sup> Control treatment (T1) had the same formulation to the ractopamine HCL treatment (T5) until Phase 5.

<sup>4</sup> EOM 1 was included at 0.02% in all 6 phases for treatments 2 and 4.

<sup>5</sup> EOM 1 was included at 0.02% for Phases 3 to 6 and EOM 2 was included at 0.01% for Phases 1 to 6 for treatment 3.

**Table 5. Chemical analysis of experimental diets (as-fed basis)<sup>1,2</sup>**

Feed additive:	Phase 6				
	Control <sup>3</sup>	EOM 1 <sup>4</sup>	EOM 1+2 <sup>5</sup>	EOM 1	Ractopamine HCL
Item, %					
DM	87.4	87.4	87.0	88.0	89.5
CP	12.7	11.7	11.9	15.3	14.1
Ca	0.46	0.55	0.48	0.62	0.64
P	0.36	0.34	0.32	0.41	0.38
Ether extract	2.5	2.5	2.8	2.8	3.0
Ash	2.8	2.7	2.9	3.4	3.5

<sup>1</sup> Multiple diet samples were collected from each diet throughout the study, homogenized, and then subsampled for analysis (Ward Laboratories, Inc. Kearney, NE).

<sup>2</sup> Phase 6 was fed from d 106 to 125.

<sup>3</sup> Control treatment (T1) had the same formulation to the ractopamine HCL treatment (T5) until phase 5.

<sup>4</sup> EOM 1 was included at 0.02% in all 6 phases for treatments 2 and 4.

<sup>5</sup> EOM 1 was included at 0.02% for Phase 3 to 6 and EOM 2 was included at 0.01% for Phase 1 to 6 for treatment 3.

**Table 6. The effects of dietary phytonics on the growth, carcass characteristics, and economics of grow-finish pigs<sup>1,2,3</sup>**

CP in Phase 6, %:	12			16		SEM	Probability, P<
Feed additive:	Control	EOM 1	EOM 1+2	EOM 1	Ractopamine HCl <sup>4</sup>		Treatment
<b>BW, lb</b>							
d 0	48.8	48.8	48.7	48.7	48.7	1.01	0.998
d 125	270.6	274.7	274.7	273.0	273.1	2.37	0.611
<b>d 0 to 125</b>							
ADG, lb	1.79	1.83	1.83	1.82	1.82	0.014	0.215
ADFI, lb	4.82 <sup>bc</sup>	4.92 <sup>ab</sup>	5.01 <sup>a</sup>	4.91 <sup>ab</sup>	4.78 <sup>c</sup>	0.046	0.003
F/G	2.68 <sup>ab</sup>	2.67 <sup>ab</sup>	2.72 <sup>a</sup>	2.69 <sup>a</sup>	2.61 <sup>b</sup>	0.026	0.047
<b>Carcass characteristics</b>							
HCW, lb	208.3 <sup>b</sup>	209.1 <sup>b</sup>	214.0 <sup>a</sup>	211.8 <sup>ab</sup>	214.6 <sup>a</sup>	1.34	0.001
Carcass ADG, lb <sup>5</sup>	1.38 <sup>c</sup>	1.39 <sup>bc</sup>	1.43 <sup>a</sup>	1.41 <sup>ab</sup>	1.43 <sup>a</sup>	0.009	0.002
Carcass F/G <sup>6</sup>	3.50 <sup>a</sup>	3.53 <sup>a</sup>	3.50 <sup>a</sup>	3.49 <sup>a</sup>	3.34 <sup>b</sup>	0.029	<0.001
Carcass yield, %	77.0 <sup>bc</sup>	76.1 <sup>c</sup>	77.9 <sup>ab</sup>	77.6 <sup>ab</sup>	78.6 <sup>a</sup>	0.53	0.021
Backfat, <sup>7</sup> in.	0.67 <sup>a</sup>	0.68 <sup>a</sup>	0.66 <sup>a</sup>	0.66 <sup>a</sup>	0.61 <sup>b</sup>	0.011	<0.001
Loin depth, <sup>7</sup> in.	2.75	2.73	2.68	2.73	2.71	0.036	0.819
Lean, <sup>7</sup> %	56.8 <sup>b</sup>	56.7 <sup>b</sup>	56.8 <sup>b</sup>	56.9 <sup>b</sup>	57.8 <sup>a</sup>	0.19	0.002
<b>Economics, \$/pig</b>							
Feed cost	54.24 <sup>c</sup>	56.21 <sup>b</sup>	58.61 <sup>a</sup>	57.28 <sup>ab</sup>	56.88 <sup>b</sup>	0.547	<0.001
Feed cost/lb gain <sup>8</sup>	0.242 <sup>c</sup>	0.246 <sup>bc</sup>	0.256 <sup>a</sup>	0.252 <sup>a</sup>	0.250 <sup>ab</sup>	0.002	0.001
Gain value <sup>9</sup>	120.22 <sup>c</sup>	120.76 <sup>bc</sup>	124.25 <sup>a</sup>	122.72 <sup>ab</sup>	124.64 <sup>a</sup>	0.851	<0.001
IOFC <sup>10</sup>	65.99 <sup>ab</sup>	64.55 <sup>b</sup>	65.64 <sup>b</sup>	65.45 <sup>b</sup>	67.77 <sup>a</sup>	0.723	0.030

<sup>1</sup> A total of 1,260 pigs (PIC 1050 × 327) were used with 28 pigs per pen and 9 replications per treatment.

<sup>2</sup> Treatment 1 was the control with 12% of CP in Phase 6 diet. Treatment 2 contained EOM 1 fed all phases with 12% of CP in Phase 6 diet. Treatment 3 was EOM 1 fed from Phase 3 to 6 and EOM 2 fed all phases with 12% CP in Phase 6. Treatment 4 contained EOM 1 fed all 6 phases with 16% CP in Phase 6. Treatment 5 contained ractopamine HCL (9 g/ton) with 16% CP in Phase 6 diet.

<sup>3</sup> Means with the same letter are not significantly different from each other.

<sup>4</sup> Paylean (Elanco Animal Health, Greenfield, IN).

<sup>5</sup> Carcass average daily gain = overall ADG \* carcass yield.

<sup>6</sup> Carcass F/G = overall average feed intake/carcass average daily gain.

<sup>7</sup> Adjusted using HCW as a covariate.

<sup>8</sup> Feed cost/lb gain = total feed cost divided by total gain per pig.

<sup>9</sup> Gain value = (HCW × \$0.70) - (d 0 BW × 0.75 × \$0.70).

<sup>10</sup> Income over feed cost = gain value – feed cost.

## Influence of Chromium Dose and Feeding Regimen on Growth Performance and Carcass Composition of Pigs Housed in a Commercial Environment<sup>1,2</sup>

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### Summary

A study was conducted to determine the effects of increasing chromium propionate (KemTRACE Cr; Kemin Industries Inc., Des Moines, IA) and feeding regimen on growth performance and carcass characteristics of finishing pigs housed in a commercial environment. There were a total of 1,206 pigs (PIC 337 × 1050; initial BW = 63.2 lb) with 27 pigs/pen and 9 pens/treatment. Pigs were split by gender upon arrival at the facility, with 4 blocks of each gender and a final mixed gender block. Gender blocks were randomly allotted to groups of 5 pen locations within the barn. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in a 5-phase feeding program. Treatments were arranged as a 2 × 2 + 1 factorial with a control diet containing no added Cr, or diets containing either 100 or 200 ppb of Cr fed during the grower (dietary Phases 1 and 2; 63 to 138 lb BW) and/or finisher (dietary Phases 3, 4, and 5; 138 to 307 lb BW) periods. For growth performance, there was no effect of changing Cr supplementation between the growing and finishing periods. Therefore, only linear and quadratic effects of increasing Cr within growth period were considered using all treatments, as well as linear and quadratic effects of the 3 treatments fed increasing Cr for the full duration of the study. Increasing Cr during the grower period decreased (quadratic,  $P < 0.001$ ) ADG and worsened F/G. During the finisher period, increasing Cr tended (quadratic,  $P = 0.061$ ) to improve F/G, with the best F/G observed in pigs fed 100 ppb. Overall, increasing Cr had no impact on ADG or ADFI; however, F/G was optimized (quadratic,  $P = 0.018$ ) when pigs were fed 100 ppb of added Cr. Carcass characteristics were not influenced by added Cr level or Cr feeding regimen. In summary, increasing dietary Cr supplementation elicited minor changes in growth performance with the best F/G observed with 100 ppb of added Cr.

<sup>1</sup> Appreciation is expressed to New Horizon Farms (Pipestone, MN) for providing the animals and research facilities, and to H. Houselog, M. Heintz, and C. Steck for technical assistance.

<sup>2</sup> Appreciation is expressed to Kemin Industries (Des Moines, IA) for project funding.

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Key words: chromium propionate, duration, finishing pig, level

## Introduction

Research evaluating the impacts of supplemental Cr in finishing pig diets has been conducted since the mid 1990's. Evidence has shown Cr plays a role in carbohydrate, lipid, protein, and nucleic acid metabolism.<sup>5,6</sup> In addition, Cr is associated with insulin sensitivity in the form of glucose tolerance factor.<sup>7</sup> The results of many of these past studies were highly variable in regards to animal performance and carcass composition. Corn-soybean meal-based diets contain a significant amount of Cr ranging from 1,000 to 3,000 ppb but are thought to have a lower bioavailability relative to other forms of chromium.<sup>8</sup> Due to the variability in ingredient chromium levels and inconsistent performance, there is currently no quantitative estimate for Cr requirements for swine.<sup>5</sup>

Recently, a meta-analysis was conducted including 31 different studies that evaluated Cr supplementation in finishing pig diets. The analysis observed that improvements in growth performance (ADG and F/G) and carcass composition (reduced backfat and increased percentage lean) can be expected with Cr supplementation.<sup>9</sup> The meta-analysis also suggested that chromium dosage and supplementation duration could affect the degree of improvement observed. This would suggest feeding strategies that combine Cr dosage and feeding duration could be optimized to achieve the greatest overall benefit. Therefore, the objective of this experiment was to determine the effects of Cr dosage and feeding regimen on growth performance and carcass composition of pigs housed in a commercial environment.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research-finishing site in southwest Minnesota. The barn was naturally ventilated and double-curtain-sided. Each pen (18 × 10 ft) was equipped with a 4-hole stainless steel feeder and cup waterer for ad libitum access to feed and water and allowed approximately 6.5 ft<sup>2</sup>/pig. Hourly ambient barn temperatures were recorded throughout the experiment (EasyLog Data Loggers; Lascar Electronics, Erie, PA). Feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 1,206 pigs (PIC 337 × 1050; initial BW = 63.2 lb) were used in a 125-d growth trial with 27 pigs/pen and 9 pens/treatment. Pigs were split by gender upon arrival at the facility, with 4 blocks of each gender and a final mixed sex gender block. Gender blocks were randomly allotted to groups of 5 pen locations within the barn. Diets were corn-soybean meal-based and fed in meal form, with dietary phases formulated for 60 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb BW ranges.

<sup>5</sup> NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, D.C..

<sup>6</sup> Trace Elements in Human and Animal Nutrition. 1977. 4<sup>th</sup> ed. Academic Press Inc., New York, NY.

<sup>7</sup> Swine Nutrition. 2001. 2<sup>nd</sup> ed. CRC Press LLC, Boca Raton, FL.

<sup>8</sup> Lindemann, M. D., 2007. Use of chromium as an animal feed supplement. In: J. B. Vincent, editor, The Nutritional Biochemistry of Chromium (III). Elsevier, Amsterdam. p. 85–118.

<sup>9</sup> Sales, J., and F. Jancik. 2011. Effects of dietary chromium supplementation on performance, carcass characteristics, and meat quality of growing-finishing swine: A meta-analysis. J. Anim. Sci. 89: 4054-4067.

All nutrients were formulated to meet or exceed the NRC (2012) requirement estimates within phases. The treatment phases were divided into two specific growth ranges including a grower period (dietary Phases 1 and 2) and a finisher period (dietary Phases 3, 4, and 5). Treatments were arranged as a  $2 \times 2 + 1$  factorial with a control diet containing no added Cr or as diets containing either 100 or 200 ppb of Cr (KemTRACE Cr; Kemin Industries Inc., Des Moines, IA) fed during the grower (dietary Phase 1 and 2; 63 to 138 lb BW) and/or finisher (dietary Phase 3 to 5; 138 to 307 lb) periods. Thus, three treatments received the same level of Cr supplementation throughout the entire trial (0, 100, or 200 ppb added Cr), while two treatments alternated supplementation levels between the grower and finisher period (100 ppb in grower followed by 200 ppb in finisher or 200 ppb in grower followed by 100 ppb in finisher, respectively). Three diets per phase were manufactured and supplemented with 0, 100, or 200 ppb Cr at a commercial feedmill (New Horizon Feeds, Pipestone, MN; Table 1) and were fed to the respective pens. Ractopamine HCl (Paylean 9 g/ton; Elanco Animal Health, Greenfield, IN) was included in Phase 5 diets and was fed for 38 d.

Samples of the complete feed were taken from the feeder at the beginning and end of each phase. Subsamples of each diet were then submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and Cr analysis (University of Guelph Agriculture & Food Laboratory; Guelph, ON). Pens of pigs were weighed and feeder measurements were recorded at the time of dietary phase changes, first marketing, and conclusion of the trial (d 0, 20, 39, 53, 87, 97, and 125) to determine ADG, ADFI, and F/G. The 3 largest pigs/pen were selected and marketed at an average barn weight of 256 lb on d 97 following the routine farm protocol with no carcass data collected on these animals. At the conclusion of the trial (d 125), the remaining animals were given a tattoo corresponding to pen number and were transported to a commercial packing facility (JBS Swift and Company; Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included live pen weight, HCW, backfat, percentage carcass lean, and loin depth. Additionally, percentage yield was calculated by dividing HCW by mean animal live plant weight for the corresponding pen.

Data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Block was included in the model as a random effect and accounted for gender, location within barn, and initial BW at the time of allotment. Linear and quadratic effects of increasing Cr within growth period were considered using all treatments, as well as linear and quadratic effects of increasing Cr fed at a constant level for the full duration of the trial. An additional contrast was analyzed to determine the impact of changing Cr concentrations between the grower and finisher periods. Backfat, loin depth, and percentage lean were adjusted to a common carcass weight for analysis using HCW as a covariate. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ .

## Results and Discussion

As expected, chemical analysis of complete diets revealed no notable differences among treatments (Tables 2 to 4). The analyzed level of dietary Cr generally followed the basal target addition rates.

The crossover contrast did not indicate any differences ( $P > 0.446$ ) in performance (ADG, ADFI, F/G, and carcass characteristics) among treatments changing Cr supplementation levels between the grower and finisher periods. This indicated there was no benefit associated with changing supplementation levels between growth periods. Because no differences for this contrast were observed, only linear and quadratic effects of increasing Cr within growth period were considered using all treatments, as well as linear and quadratic effects of increasing Cr for the full duration using the 3 treatments that had a constant Cr supplementation level throughout.

Increasing Cr during the grower period reduced (quadratic,  $P < 0.001$ ; Table 5) ADG and worsened F/G. During the finisher period, increasing Cr tended (quadratic,  $P = 0.061$ ) to improve F/G with the best F/G observed in pigs fed 100 ppb of added Cr. Overall, increasing Cr had no impact on ADG or ADFI ( $P > 0.05$ ); however, F/G was optimized (quadratic,  $P = 0.018$ ) when pigs were fed 100 ppb added Cr. Carcass characteristics were not influenced by added Cr level or feeding regimen.

Meta-analysis conducted by Sales and Jancik (2011) attempted to summarize and quantify the effect of dietary chromium supplementation on carcass characteristics of finishing swine, as the variability among studies is quite significant. Their evaluation included studies which supplemented chromium in the form of Cr Met chelate, Cr nanocomposite, Cr nicotinate, Cr propionate, Cr tripicolinate, and Cr yeast. The analysis would suggest a reduction ( $P < 0.05$ ) in 10th-rib backfat thickness and an increase in percentage lean and loin muscle area (Hedge's  $g$  standardized effect size = -0.416, 0.491, and 0.494, respectively). However, these differences in carcass composition were not observed with the current study. Additionally, the meta-analysis would suggest an improvement ( $P < 0.05$ ) in ADG and G:F would be expected with Cr supplementation (Hedge's  $g$  standardized effect size = 0.149 and 0.302, respectively), which was not observed in the current study.

In summary, increasing dietary Cr supplementation elicited minor changes in growth performance in our current study, with the best F/G observed with 100 ppb of added Cr.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Dietary phase				
	1	2	3	4	5
Ingredient, %					
Corn	56.00	61.25	65.80	69.25	67.25
Soybean meal, 46.5% CP	21.65	16.50	12.00	8.55	20.65
DDGS <sup>2</sup>	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	1.25	1.28	1.23	1.20	1.03
Monocalcium phosphate, 21% P	0.15	---	---	---	0.10
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.36	0.37	0.39	0.39	0.28
DL-Met	0.01	---	---	---	0.04
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	---	0.01	0.02	0.02	---
Ractopamine <sup>3</sup>	---	---	---	---	0.03
Phytase <sup>4</sup>	0.01	0.01	0.01	0.01	0.01
Trace mineral premix	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.08	0.08	0.08	0.08	0.08
KemTRACE Cr <sup>5</sup>	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100

*continued*

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Dietary phase				
	1	2	3	4	5
Calculated analysis <sup>6</sup>					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.02	0.91	0.82	0.74	0.90
Ile:Lys	63	62	60	59	64
Leu:Lys	152	159	164	171	150
Met:Lys	29	29	30	31	32
Met and Cys:Lys	55	56	57	59	59
Thr:Lys	61	61	61	63	65
Trp:Lys	18.4	18.4	18.4	18.4	19.0
Val:Lys	70	70	70	70	71
Total Lys, %	1.19	1.06	0.96	0.87	1.04
ME, kcal/lb	1,502	1,506	1,509	1,511	1,506
NE, kcal/lb	1,102	1,118	1,130	1,140	1,123
SID Lys:ME, g/Mcal	3.08	2.74	2.46	2.22	2.71
SID Lys:NE, g/Mcal	4.20	3.69	3.29	2.94	3.64
CP, %	20.0	18.1	16.4	15.1	17.6
Ca, %	0.61	0.57	0.54	0.52	0.50
P, %	0.45	0.40	0.38	0.36	0.40
Available P, %	0.29	0.26	0.25	0.25	0.24

<sup>1</sup>Diets were fed in a 5-phase feeding program formulated to 60 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb BW ranges.

<sup>2</sup>DDGS = dried distillers grains with solubles.

<sup>3</sup>Paylean 9 g/lb (Elanco, Greenfield, IN).

<sup>4</sup>Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.11% available P.

<sup>5</sup>KemTRACE Cr (chromium propionate; Kem Industries Inc., Des Moines, IA) was added at 0.5 lb/ton (100 ppb Cr) or 1.0 lb/ton (200 ppb Cr) at the expense of corn.

<sup>6</sup>NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

**Table 2. Chemical analysis of diets, Phases 1 and 2 (as-fed basis)<sup>1,2</sup>**

Item	Added Cr, ppb:	Phase 1			Phase 2		
		0	100	200	0	100	200
DM, %		88.08	88.73	88.52	85.1	89.02	89.02
CP, %		19.4	17.9	20.0	18.8	15.3	20.2
Ether extract, %		3.1	2.9	3.4	4.6	3.6	3.6
Crude fiber, %		3.3	3.1	3.3	3.2	3.1	3.7
Cr, ppb		590	600	790	540	610	710

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

<sup>2</sup>Phase 1 was fed from approximately 60 to 100 lb and Phase 2 fed from approximately 100 to 135 lb.

**Table 3. Chemical analysis of diets, Phases 3 and 4 (as-fed basis)<sup>1,2</sup>**

Item	Added Cr, ppb:	Phase 3			Phase 4		
		0	100	200	0	100	200
DM, %		88.57	88.55	88.69	88.67	88.22	89.11
CP, %		19.5	16.9	15.2	15.1	14.5	14.1
Ether extract, %		3.6	3.8	3.7	3.8	3.9	3.8
Crude fiber, %		3.4	3.1	3.2	3.0	3.0	3.2
Cr, ppb		500	430	590	480	490	620

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

<sup>2</sup>Phase 3 was fed from approximately 135 to 170 lb and Phase 4 fed from approximately 170 to 235 lb.

**Table 4. Chemical analysis of diets, Phase 5 (as-fed basis)<sup>1,2</sup>**

Item	Added Cr, ppb:	Phase 5		
		0	100	200
DM, %		88.92	88.27	88.67
CP, %		17.3	16.6	17.7
Ether extract, %		3.1	3.0	2.9
Crude fiber, %		2.6	2.6	3.0
Cr, ppb		430	480	610

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

<sup>2</sup>Phase 5 was fed from approximately 230 to 280 lb.

**Table 5. Effects of added chromium on finishing pig growth and carcass characteristics<sup>1,2,3</sup>**

Grower added Cr, ppb:	0	100	200	100	200	SEM	Probability, <i>P</i> <	
							Linear <sup>4</sup>	Quadratic <sup>4</sup>
Finisher added Cr, ppb:	0	100	200	200	100			
<b>BW, lb</b>								
d 0	63.3	63.0	63.4	63.0	63.2	1.03	0.955	0.720
d 39	140.1	139.8	135.2	141.3	134.1	1.57	0.001	0.005
d 125	306.4	308.5	305.7	309.0	307.3	2.99	0.824	0.354
<b>Grower (d 0 to 39)</b>								
ADG, lb	1.97	1.97	1.84	2.01	1.82	0.026	0.001	0.001
ADFI, lb	3.91	3.90	3.85	3.93	3.84	0.062	0.229	0.341
F/G	1.99	1.98	2.10	1.96	2.11	0.025	0.001	0.001
<b>Finisher (d 39 to 125)</b>								
ADG, lb	1.65	1.67	1.68	1.66	1.71	0.026	0.369	0.106
ADFI, lb	5.40	5.34	5.37	5.38	5.42	0.099	0.656	0.860
F/G	3.28	3.20	3.21	3.23	3.17	0.052	0.208	0.061
<b>Overall (d 0 to 125)</b>								
ADG, lb	1.96	1.99	1.96	1.98	1.97	0.020	0.796	0.136
ADFI, lb	4.91	4.87	4.88	4.91	4.91	0.083	0.472	0.651
F/G	2.50	2.45	2.49	2.48	2.49	0.027	0.507	0.018
<b>Carcass characteristics<sup>5</sup></b>								
HCW, lb	224.2	226.0	222.6	225.8	224.4	2.03	0.404	0.136
Backfat, in	0.642	0.641	0.643	0.644	0.641	0.0218	0.943	0.924
Lean, %	57.33	57.44	57.34	57.43	57.44	0.385	0.974	0.665
Loin depth, in	2.76	2.80	2.77	2.79	2.79	0.029	0.611	0.307
Yield, %	73.21	73.27	72.80	73.09	73.01	0.238	0.235	0.370

<sup>1</sup> A total of 1,206 finisher pigs (initially 63.2 lb BW) were used in a 125-d finisher study with 27 pigs per pen and 9 replications per treatment. Pigs were fed in split gender pens, with 4 replicates per gender and 1 mixed gender replicate. Gender, weight, and location served as blocking factors in allotment to treatment. Diets were fed in a 5-phase feeding program formulated to 60 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb BW ranges.

<sup>2</sup> Treatment diets were fed in two growth stages, grower (Phases 1-2) and finisher (Phases 3-5) and were supplemented with 0, 100, or 200 ppb chromium propionate (KemTRACE Cr; Kemin Industries Inc., Des Moines, IA).

<sup>3</sup> Linear and quadratic contrasts were made for the grower, finisher, and full trial periods. The full contrast statement only included treatments which received the same level of Cr supplementation for the full duration of the experiment. The two treatments having a crossover structure between the grower and finisher phases were analyzed with a crossover contrast to compare these two treatments for the full trial period. The crossover contrast was used for overall ADG, ADFI, and F/G, as well as carcass characteristics and was not significant (*P* > 0.446).

<sup>4</sup> Grower linear and quadratic contrasts were used for d 39 BW, grower period ADG, ADFI, and F/G. Finisher linear and quadratic contrasts were used for finisher period ADG, ADFI, and F/G. Full trial linear and quadratic contrast statements were used for d 0 BW, d 125 BW, overall ADG, ADFI, F/G, and carcass characteristics.

<sup>5</sup> Backfat, percentage lean, and loin depth were analyzed by adjusting for a common HCW.

## Determining the Influence of KemTRACE Cr and/or Micro-Aid on Growth Performance and Carcass Composition of Pigs Housed in a Commercial Environment<sup>1,2</sup>

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### Summary

A study was conducted to determine the interactive effects of chromium propionate (KemTRACE Cr; Kemin Industries Inc., Des Moines, IA) and Micro-Aid (*Yucca schidigera*-based product; Distributors Processing Inc., Porterville, CA) on growth performance and carcass composition of finishing pigs housed in a commercial environment. There were a total of 1,188 pigs (PIC 337 × 1050; initial BW = 60.3 lb) with 27 pigs/pen and 11 pens/treatment. Pigs were split by gender upon arrival at the facility, with 5 blocks of each gender and a final mixed sex gender block. Gender blocks were randomly allotted to groups of 4 pen locations within the barn. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in 5 phases. All nutrients were formulated to meet or exceed NRC (2012) requirement estimates. Treatments were arranged as a 2 × 2 factorial with main effects of Cr (0 vs 200 ppb) or Micro-Aid (0 vs 62.5 ppm). There were no Cr × Micro-Aid interactions observed for growth or carcass measurements. Overall, ADG and F/G were not influenced by treatment. Adding Cr alone increased ( $P = 0.048$ ) ADFI, and inclusion of Micro-Aid resulted in a marginally significant increase ( $P = 0.076$ ) in ADFI. For carcass characteristics, HCW, loin depth, and percentage carcass yield were not influenced by treatment. Backfat depth tended to increase ( $P = 0.055$ ) and lean percentage was decreased ( $P = 0.014$ ) when Cr was added to diets. In summary, no synergistic effects were observed from feeding Cr and Micro-Aid in diets fed to finishing pigs housed in a commercial environment. Only marginal differences were observed from adding Cr or Micro-Aid with increased ADFI observed from feeding either. Finally, diets containing added Cr tended to be associated

<sup>1</sup> Appreciation is expressed to New Horizon Farms (Pipestone, MN) for providing the animals and research facilities and to H. Houselog, M. Heintz, and C. Steck for technical assistance.

<sup>2</sup> Appreciation is expressed to Kemin Industries (Des Moines, IA) for project funding.

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with carcasses having more backfat and less lean suggesting the increased ADFI was not utilized for increased muscle deposition.

Key words: chromium propionate, Micro-Aid, pigs

## Introduction

Chromium plays a role in carbohydrate, lipid, protein, and nucleic acid metabolism.<sup>5,6</sup> In addition, Cr is associated with insulin sensitivity in the form of a cofactor for glucose tolerance factor.<sup>7</sup> Corn-soybean meal-based diets contain a significant amount of Cr ranging from 1,000 to 3,000 ppb, but much of that is thought to be unavailable to the animal.<sup>8</sup> Recently, a meta-analysis was conducted including 31 different research studies that evaluated Cr supplementation in finishing pig diets. They observed that improvements in growth performance (ADG and F/G) and carcass composition (reduced backfat and increased percentage lean) can be expected with Cr supplementation.<sup>9</sup>

Additionally, *Yucca schidigera* is believed to have a positive impact on gastrointestinal microflora through its saponin characteristics thereby reducing gaseous emissions and potentially improving growth performance; however, limited research exists.<sup>10</sup> Research evaluating the effects of *Yucca schidigera* supplementation in poultry is available, and would suggest an improvement in F/G.<sup>11</sup> Additionally, research in mice with artificially induced diabetes mellitus indicates a potential reduction of circulating glucose levels when supplemented with *Yucca schidigera* extract through a potential insulin releasing mechanism from pancreatic  $\beta$ -cells.<sup>12</sup> Research related to the impact of *Yucca schidigera* on blood metabolites in swine is currently very limited, and there are no data available to determine the interactive effects of Cr and *Yucca schidigera* when fed to finishing pigs. Therefore, the objective of this experiment was to determine the effects of Cr supplementation with and without Micro-Aid on growth performance and carcass composition of pigs housed in a commercial environment.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research-finishing site in southwest Minnesota. The barn was naturally ventilated and double-curtain-sided. Each pen (18 × 10 feet) was equipped with a 4-hole stainless steel feeder and cup waterer for ad libitum access to feed and water and allowed approximately 6.5 ft<sup>2</sup>/pig. Hourly ambient barn temperatures were recorded throughout the ex-

<sup>5</sup> NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, D.C.

<sup>6</sup> Trace Elements in Human and Animal Nutrition. 1977. 4<sup>th</sup> ed. Academic Press Inc., New York, NY.

<sup>7</sup> Swine Nutrition. 2001. 2<sup>nd</sup> ed. CRC Press LLC, Boca Raton, FL.

<sup>8</sup> Lindemann, M. D., 2007. Use of chromium as an animal feed supplement. In: J. B. Vincent, editor, The Nutritional Biochemistry of Chromium (III). Elsevier, Amsterdam. p. 85–118.

<sup>9</sup> Sales, J., and F. Jancik. 2011. Effects of dietary chromium supplementation on performance, carcass characteristics, and meat quality of growing-finishing swine: A meta-analysis. J. Anim. Sci. 89: 4054-4067.

<sup>10</sup> Colina, J.J., A.J. Lewis, P.S. Miller, and R.L. Fischer. 2001. Dietary manipulation to reduce aerial ammonia concentrations in nursery pig facilities. J. Anim. Sci. 79: 3096-3103.

<sup>11</sup> Sahoo S.P., D. Kaur, A.P. Sethi, A. Sharma, and M. Chandra. 2015. Evaluation of *Yucca schidigera* extract as feed additive on performance of broiler chicks in winter season. Veterinary World 8(4): 556-560.

<sup>12</sup> Oztasan, N. 2013. The effects of *Yucca schidigera* on blood glucose and lipid levels in diabetic rats. African Journal of Biochemistry Research. 7(9): 179-183.

periment (EasyLog Data Loggers; Lascar Electronics, Erie, PA). Feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 1,188 pigs (PIC 337 × 1050; initial BW = 60.3 lb) with 27 pigs/pen and 11 pens/treatment were used in a 117-d study. Pens were blocked by BW and were randomly assigned to diets with 27 pigs per pen and 7 pens per treatment. Pigs were split by gender upon arrival at the facility, with 5 blocks of each gender and a final mixed sex gender block. Gender blocks were randomly allotted to groups of 4 pen locations within the barn. Diets were corn-soybean meal-dried distillers grains with solubles-based and were fed in 5 phases. All nutrients were formulated to meet or exceed NRC (2012) requirement estimates (Table 1). Treatments were arranged in a 2 × 2 factorial with main effects of Cr (0 vs. 200 ppb; KemTRACE Cr; Kemin Industries Inc., Des Moines, IA) or Micro-Aid (0 vs. 62.5 ppm; Micro-Aid (a *Yucca schidigera*-based product); Distributors Processing Inc., Porterville, CA) and were fed for the full duration of the experiment. Ractopamine HCl (Paylean 9 g/ton; Elanco Animal Health, Greenfield, IN) was included in phase 5 diets and was fed for 27 d. The 4 experimental diets were manufactured at a commercial feedmill (New Horizon Feeds, Pipestone, MN).

Samples of the complete feed were taken from the feeder at the beginning and end of each phase and diet samples were subsampled using a riffle-splitter, then submitted for proximate (Ward Laboratories, Inc., Kearney, NE) and chromium analysis (University of Guelph Agriculture & Food Laboratory; Guelph, ON). Pens of pigs were weighed and feeder measurements were recorded at the time of dietary phase changes, first marketing, and conclusion of the trial (d 0, 21, 39, 59, 90, 97, and 117) to determine ADG, ADFI, and F/G. The 3 largest pigs/pen were selected and marketed at an average barn weight of 245 lb on d 97 and marketed following the routine farm protocol with no carcass data collected on these animals. At the conclusion of the trial (d 117), remaining animals were given a tattoo corresponding to pen and were transported to a commercial packing facility (JBS Swift and Company; Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included live pen weight, HCW, backfat, percentage carcass lean, and loin depth. Additionally, percentage yield was calculated by dividing HCW by average live animal plant weight for the corresponding pen.

Data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Block was included in the model as a random effect and accounted for gender, location within barn, and initial BW at the time of allotment. Backfat, loin depth, and percentage lean were adjusted to a common carcass weight for analysis using HCW as a covariate. Results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ .

## Results and Discussion

As expected, chemical analysis of complete diets revealed no notable differences among treatments (Tables 2 to 4). The only discrepancy was in Phase 3 when both Cr and Micro-Aid were included and the dietary level of Cr was lower than expected.

There were no Cr × Micro-Aid interactions observed for the entire study (Table 5). For the grower period, added Cr increased ( $P < 0.028$ ) ADG and ADFI. Added Micro-Aid in the grower period tended to worsen ( $P = 0.051$ ) F/G. During the finishing period, added Cr tended to increase ( $P = 0.080$ ) ADFI but worsen F/G. Added Micro-Aid in the finishing period tended to increase ( $P = 0.088$ ) ADFI. Overall, ADG and F/G were not influenced by treatment. Adding Micro-Aid tended to increase ( $P = 0.076$ ) and adding Cr increased ( $P = 0.048$ ) ADFI. For carcass characteristics, HCW, loin depth, and carcass yield were not influenced by treatment. Backfat depth tended to increase ( $P = 0.055$ ) and lean percentage decreased ( $P = 0.014$ ) when Cr was added into the diets.

Meta-analysis conducted by Sales and Jancik (2011) suggests a reduction ( $P < 0.05$ ) in 10th-rib backfat thickness and increase in percentage lean (Hedge's  $g$  standardized effect size = -0.416 and 0.491, respectively) when finishing pigs are supplemented with dietary Cr. Results from the current study did not support this conclusion, and an increase in backfat thickness leading to a reduction in percentage lean was observed. Colina et al. (2001) did not observe any differences ( $P \geq 0.41$ ) in ADG, ADFI, or G/F when *Yucca schidigera* was included in nursery pig diets at a level of 125 ppm. Additionally, the advantages observed with *Yucca schidigera* supplementation in broiler production by Sahoo et al. (2015) do not appear to be transferable to nursery or finishing pig production, with little research currently available.

In summary, no synergistic effects were observed from feeding Cr and Micro-Aid in diets fed to finishing pigs housed in a commercial environment. Only marginal differences were observed from adding either Cr or Micro-Aid with increased ADFI observed from feeding either Cr or Micro-Aid. Finally, diets containing added Cr tended to be associated with carcasses having more backfat and less lean. This suggests the increased ADFI observed from pigs fed added Cr was not utilized to support lean deposition, but rather was converted to backfat.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Dietary phase				
	1	2	3	4	5
Ingredient, %					
Corn	56.05	61.25	65.80	69.25	67.25
Soybean meal, 46.5% CP	21.60	16.50	11.95	8.55	20.65
DDGS <sup>2</sup>	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	1.25	1.28	1.23	1.20	1.03
Monocalcium phosphate, 21% P	0.15	---	---	---	0.10
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.36	0.37	0.39	0.39	0.28
DL-Met	0.01	---	---	---	0.04
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	---	0.01	0.02	0.02	---
Ractopamine HCl <sup>3</sup>	---	---	---	---	0.03
Phytase <sup>4</sup>	0.01	0.01	0.01	0.01	0.01
Trace mineral premix	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.08	0.08	0.08	0.08	0.08
KemTRACE Cr <sup>5</sup>	+/-	+/-	+/-	+/-	+/-
Micro-Aid <sup>6</sup>	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100

*continued*

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Dietary phase				
	1	2	3	4	5
Calculated analysis <sup>4</sup>					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.02	0.91	0.82	0.74	0.90
Ile:Lys	63	62	60	59	64
Leu:Lys	152	159	164	171	150
Met:Lys	29	29	30	31	32
Met and Cys:Lys	55	56	57	59	59
Thr:Lys	61	61	61	63	65
Trp:Lys	18.4	18.4	18.4	18.4	19.0
Val:Lys	70	70	70	70	71
Total Lys, %	1.18	1.06	0.96	0.87	1.04
ME, kcal/lb	1,502	1,506	1,509	1,511	1,506
NE, kcal/lb	1,103	1,118	1,130	1,140	1,123
SID Lys:ME, g/Mcal	3.08	2.74	2.46	2.22	2.71
SID Lys:NE, g/Mcal	4.20	3.69	3.29	2.94	3.64
CP, %	20.0	18.1	16.4	15.0	17.6
Ca, %	0.61	0.57	0.54	0.52	0.50
P, %	0.45	0.40	0.38	0.36	0.40
Available P, %	0.29	0.26	0.25	0.25	0.24

<sup>1</sup>Treatment diets were fed for the full duration of the trial and were formulated for 60 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb BW ranges.

<sup>2</sup>DDGS = dried distillers grains with solubles.

<sup>3</sup>Paylean 9 g/lb (Elanco, Greenfield, IN).

<sup>4</sup>Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.11% available P.

<sup>5</sup>KemTRACE Cr (chromium propionate; Kemira Industries Inc., Des Moines, IA) was added at 1.0 lb/ton (200 ppb Cr) at the expense of corn in the appropriate treatment diets.

<sup>6</sup>Micro-Aid (*Yucca schidigera*-based product; Distributors Processing Inc., Porterville, CA) was added at 2.0 lb ton (62.5 ppm active ingredient) at the expense of corn in the appropriate treatment diets.

<sup>7</sup>NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, D.C.

**Table 2. Chemical analysis of diets, Phases 1 and 2 (as-fed basis)<sup>1,2</sup>**

	Phase 1				Phase 2			
	0	200	0	200	0	200	0	200
Added Cr, ppb <sup>3</sup> :	0	200	0	200	0	200	0	200
Micro-Aid, ppm <sup>4</sup> :	0	0	62.5	62.5	0	0	62.5	62.5
DM, %	88.01	87.11	88.67	88.32	88.05	88.77	88.18	87.44
CP, %	19.9	17.8	20.0	18.5	16.8	18.2	21.4	19.2
Ether extract, %	3.7	3.2	3.6	3.5	4.3	3.6	3.7	3.3
Crude fiber, %	3.6	3.6	3.0	2.8	3.4	3.5	3.5	4.6
Cr, ppb	570	710	700	910	560	730	610	800

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

<sup>2</sup> Phase 1 was fed from approximately 60 to 100 lb and Phase 2 fed from approximately 100 to 135 lb.

<sup>3</sup> KemTRACE Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

<sup>4</sup> Micro-Aid (*Yucca schidigera*-based product; Distributors Processing, Inc., Porterville, CA).

**Table 3. Chemical analysis of diets, Phases 3 and 4 (as-fed basis)<sup>1,2</sup>**

	Phase 3				Phase 4			
	0	200	0	200	0	200	0	200
Added Cr, ppb <sup>3</sup> :	0	200	0	200	0	200	0	200
Micro-Aid, ppm <sup>4</sup> :	0	0	62.5	62.5	0	0	62.5	62.5
DM, %	88.86	88.74	88.51	89.25	88.27	88.27	88.57	88.72
CP, %	16.7	16.2	16.9	15.5	15.1	15.2	15.4	14.9
Ether extract, %	3.8	3.8	3.8	3.7	3.7	3.6	3.8	3.6
Crude fiber, %	3.2	3.0	3.3	3.1	3.1	3.2	3.1	2.9
Cr, ppb	700	510	770	460	450	560	440	640

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

<sup>2</sup> Phase 3 was fed from approximately 135 to 170 lb and Phase 4 fed from approximately 170 to 235 lb.

<sup>3</sup> KemTRACE Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

<sup>4</sup> Micro-Aid (*Yucca schidigera*-based product; Distributors Processing, Inc., Porterville, CA).

**Table 4. Chemical analysis of diets, Phase 5 (as-fed basis)<sup>1,2</sup>**

	Phase 5			
	0	200	0	200
Added Cr, ppb <sup>3</sup> :	0	200	0	200
Micro-Aid, ppm <sup>4</sup> :	0	0	62.5	62.5
DM, %	87.56	88.87	88.73	88.25
CP, %	17.6	17.8	17.5	19.5
Ether extract, %	3.0	3.2	3.1	3.0
Crude fiber, %	2.6	2.9	2.8	2.8
Cr, ppb	550	660	450	580

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

<sup>2</sup> Phase 5 was fed from approximately 230 to 280 lb.

<sup>3</sup> KemTRACE Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

<sup>4</sup> Micro-Aid (*Yucca schidigera*-based product; Distributors Processing, Inc., Porterville, CA).

**Table 5. Impact of KemTRACE<sup>2</sup> Cr and Micro-Aid<sup>3</sup> on finishing pig growth performance and carcass characteristics<sup>1</sup>**

Added Cr, ppb: <sup>2</sup> Micro-Aid, ppm: <sup>3</sup>	0	200	0	200	SEM	Probability, <i>P</i> <		
						MicroAid	Cr	MicroAid × Cr
BW, kg								
d 0	60.3	60.2	60.2	60.4	1.06	0.726	0.907	0.653
d 39	133.4	134.4	133.2	134.4	1.74	0.916	0.099	0.906
d 117	284.8	286.0	285.6	287.4	2.87	0.609	0.472	0.893
Grower (d 0 to 39)								
ADG, lb	1.87	1.90	1.87	1.90	0.023	0.713	0.026	0.989
ADFI, lb	3.82	3.89	3.86	3.94	0.073	0.224	0.028	0.980
F/G	2.04	2.05	2.07	2.07	0.019	0.051	0.774	0.997
Finisher (d 39 to 117)								
ADG, lb	1.93	1.94	1.96	1.95	0.023	0.334	0.954	0.761
ADFI, lb	5.45	5.56	5.56	5.65	0.124	0.088	0.080	0.907
F/G	2.82	2.87	2.84	2.89	0.052	0.365	0.053	0.883
Overall (d 0 to 117)								
ADG, lb	1.91	1.93	1.93	1.93	0.019	0.490	0.446	0.810
ADFI, lb	4.90	4.99	4.98	5.07	0.104	0.076	0.048	0.955
F/G	2.56	2.59	2.59	2.62	0.038	0.159	0.103	0.887
Carcass characteristics <sup>4</sup>								
HCW, lb	212.1	215.1	215.0	216.8	2.41	0.160	0.139	0.715
Backfat, in	0.668	0.696	0.679	0.690	0.0252	0.787	0.055	0.371
Lean, %	56.9	55.9	56.8	56.5	0.41	0.261	0.014	0.158
Loin depth, in	2.76	2.73	2.79	2.74	0.025	0.419	0.128	0.621
Yield, %	74.51	75.23	75.27	75.44	0.456	0.254	0.302	0.511

<sup>1</sup> A total of 1,188 finisher pigs (PIC 337 × 1050; initial BW = 60.3 lb) were used in a 117-d, five phase finisher study with 27 pigs per pen and 11 replications per treatment. Pigs were fed in split gender pens, with 5 replicates per gender, and 1 mixed sex replicate. Gender, weight, and location served as blocking factors in allotment to treatment. Treatment diets were fed for the full duration of the trial and were formulated to 60 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb BW ranges.

<sup>2</sup> KemTRACE Cr (chromium propionate; Kemin Industries Inc., Des Moines, IA).

<sup>3</sup> Micro-Aid (*Yucca schidigera*-based product; Distributors Processing, Inc., Porterville, CA).

<sup>4</sup> Backfat, percentage lean, and loin depth were analyzed by adjusting for a common HCW.

## Effects of Added Chromium and Space Allocation on Finishing Pig Performance<sup>1</sup>

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### Summary

A total of 256 pigs (Line 600 × 241, DNA Columbus, NE) were used in a 72-d trial to determine the effect of dietary chromium (chromium propionate; Kemin Industries, Des Moines, IA) and space allowance on performance and carcass characteristics of finishing pigs. Pens were blocked by initial weight and randomly assigned to treatments with 8 pigs per pen and 8 pens per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of diet (control or added chromium, 200 ppb) and 2 space allowances (9.8 ft<sup>2</sup> - normal and 6.8 ft<sup>2</sup> - restricted). Adding chromium to the diet decreased ( $P = 0.044$ ) ADG from d 56 to 72 and resulted in poorer ( $P = 0.021$ ) F/G for the overall period. Space restriction decreased ( $P < 0.001$ ) ADG and ADFI for all periods within the study and final BW, and HCW, but increased ( $P = 0.009$ ) carcass yield and decreased ( $P = 0.003$ ) backfat depth. These results indicate that chromium propionate did not improve performance when pigs were restricted in space.

Key words: chromium propionate, finishing pig, stocking density

### Introduction

Inadequate space allocation can have detrimental effects on performance, economics, and welfare of pigs. The mechanism by which performance is impaired when space allowance is reduced is not well elucidated; however, changes in stress-related hormones (e.g., cortisol, ACTH), cytokines (e.g., TNF- $\alpha$ , IL-6), and behavioral responses may lead to the decreased performance.

Chromium is an essential nutrient in human and animal nutrition. In the body, chromodulin binds chromium and is theorized as having a role in insulin signaling, prolonging kinase activity, and potentiating the effect of insulin in glucose absorption in many tissues, such as liver, spleen, and kidney. The biological mode of action of insulin is interlaced with those of GH and IGF-I, both with activities related to growth and increased lean tissue deposition in pigs. Chromium supplementation has also been

<sup>1</sup> Appreciation is expressed to Kemin Industries (Des Moines, IA) for providing the chromium used in the study.

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hypothesized to alleviate stress-related responses and improve performance and carcass characteristics of finishing pigs. The objective of this work was to evaluate the effects of chromium supplementation (from chromium propionate) on the performance and carcass characteristics of finishing pigs under two different space allocations.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility was totally enclosed and environmentally regulated. The experiment was designed with 4 treatments arranged as a factorial with main effects of diet (control vs. chromium) and two different space allowances (9.8 or 6.8 ft<sup>2</sup>/pig) with 8 pigs/pen (4 barrows and 4 gilts).

The pens were equipped with adjustable gates to allow different space allowances per pig. If a pig died or was removed from a pen during the experiment, pen size was adjusted to maintain the correct space allocation per pig. Each pen was equipped with a dry, single-sided feeder (Farmweld, Teutopolis, IL) with two 14 × 4.5 in. (length × width) feeder spaces and a 1-cup waterer. Pigs were provided ad libitum access to feed and water. Pens were located over a completely slatted concrete floor with a 4-ft pit underneath for manure storage. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen.

A total of 256 pigs (Line 600 × 241 DNA, Columbus, NE) initially 129 ± 5.5 lb were used. Pigs were allotted randomly to pens upon entry into the finisher, and the experiment lasted 72 d. Pens of pigs were balanced by initial BW and randomly allotted to 1 of 4 treatments with 8 replications per treatment. Feed was manufactured at the Kansas State University O. H. Kruse Feed Technology Innovation Center, Manhattan, KS. All pigs were fed the same corn-soybean meal-based diet in meal form (Table 1). Diets were fed in 3 phases. Chromium propionate (Kemin Industries, Des Moines, IA) was added at 1 lb/ton replacing corn in the control diets for each phase. Feed samples were taken upon manufacturing during each phase. Pigs and feeders were weighed approximately every 2 wk to calculate ADG, ADFI, and F/G. An adjusted F/G was calculated to account for the different ending BW using the following equation:  $F/G_{adj} = (274 - BW \text{ on d } 72) \times 0.005 + \text{actual F/G}$ , where 274 is the mean ending BW of the space restricted pigs.

Prior to marketing, all pigs were individually weighed and tattooed for carcass data collection. They were transported approximately 2.5 h to a commercial packing plant (Triumph Foods LLC, St. Joseph, MO). Standard carcass characteristics were measured.

Data were analyzed as a generalized blocked design with diets and space allowance as fixed effects and block as a random effect using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC), with pen serving as the experimental unit. Response criteria were tested for main effects and potential interactions between chromium and space allowance.

## Results and Discussion

There were relatively few interactions between dietary chromium and space allowance for growth performance (Table 2). From d 28 to 42, adding chromium to the diet reduced ADG (interaction,  $P = 0.014$ ) for restricted pigs while numerically increasing ADG for pigs housed with adequate space (9.8 ft<sup>2</sup>). This led to a tendency ( $P = 0.066$ ) for a similar interaction for F/G. From d 56 to 72, adding chromium to the diet reduced ADFI for pigs restricted in space, but did not influence ADFI for pigs housed at 9.8 ft<sup>2</sup>/pig (interaction,  $P = 0.033$ ).

During every 2-wk period in the experiment and for the overall trial, pigs housed at 6.8 ft<sup>2</sup>/pig had decreased ( $P < 0.01$ ) ADG and ADFI compared with pigs housed at 9.8 ft<sup>2</sup>/pig. Feed efficiency was not influenced by space allowance except when F/G was adjusted to a common BW of 274 lb. After adjustment for the difference in final BW, pigs restricted in space had poorer ( $P = 0.020$ ) F/G than pigs with 9.8 ft<sup>2</sup>/pig.

Adding chromium to the diet did not influence growth performance from d 0 to 28. Besides the interactive effects, dietary chromium also reduced ( $P = 0.044$ ) ADG during the last phase of the experiment (d 56 to 72) and tended ( $P = 0.079$ ) to reduce overall ADG resulting in poorer ( $P = 0.021$ ) overall F/G.

The effects of space on ADG and ADFI were reflected in the BW of the animals for all periods within the study. Body weight of pigs provided with 9.8 ft<sup>2</sup>/pig were 1.41% ( $P = 0.013$ ), 4.04% ( $P < 0.001$ ), and 5.26% ( $P < 0.001$ ) greater on d 14, 42, and 72, respectively, than for pigs housed at 6.8 ft<sup>2</sup>/pig. Altogether, space restriction caused a 16 lb decrease in final BW. Except for a tendency ( $P = 0.056$ ) for a reduction in final BW at day 72, no effects of dietary chromium were observed for BW.

The addition of dietary chromium did not affect carcass criteria, except for a tendency ( $P = 0.069$ ) to increase backfat depth. The effects of space were more pronounced, where pigs restricted in space had decreased ( $P < 0.001$ ) HCW and backfat depth ( $P = 0.003$ ), but had increased ( $P = 0.009$ ) carcass yield and a tendency ( $P = 0.060$ ) for increased percentage lean.

Restricting space from 9.8 to 6.8 ft<sup>2</sup>/pig caused a detrimental effect on performance of finishing pigs throughout the experiment. Using broken line analysis to identify a critical  $k$  value at which production starts to decline due to space restriction, Street and Gonyou (2008)<sup>4</sup> reported a break point of  $k = 0.036$  and Gonyou et al. (2006)<sup>5</sup> reported a  $k$  of 0.034. Using these  $k$  values as a reference, for the weight range of the animals in this experiment, the  $k$  value for the first 14 d of trial was 0.0365 with 6.8 ft<sup>2</sup>/pig, which is just above the break point reported by the Street and Gonyou (2008) and Gonyou et al. (2006). Growth rate was reduced during the first 14 d prior to when pigs should not

<sup>4</sup> Street, B. R. and Gonyou, H. W., 2008. Effects of housing finishing pigs in two group sizes and at two floor space allocations on production, health, behavior, and physiological variables. *J. Anim. Sci.* 86: 982–991.

<sup>5</sup> Gonyou, H. W., M. C. Brumm, E. Bush, J. Deen, S. A. Edwards, T. Fangman, J. J. McGlone, M. Meunier-Salaun, R. B. Morrison, H. Spooler, P. L. Sundberg, and A. K. Johnson. 2006. Application of broken-line analysis to assess floor space requirements of nursery and grower-finisher pigs expressed on an allometric basis. *J. Anim. Sci.* 84: 229-235.

have had their growth restricted due to space. For all the other periods, the  $k$  values are all below 0.036, with values reaching as low as 0.0246 when pigs were between 245 and 280 lb. Pigs provided 9.8 ft<sup>2</sup>/pig on the other hand, just barely reach the 0.036 mark at the end of the last period (0.0352).

Dietary chromium was not able to attenuate the negative effects of the stress caused by restricted space. In fact, it seems chromium further decreased performance of the pigs when they were restricted in space, as final BW of chromium/restricted-space pigs was reduced by 4 lb (271.8 lb) when compared to control pigs that were restricted in space (276.1 lb).

**Table 1. Composition of experimental diets (as-fed basis)**

Ingredient %	Control diet		
	Phase 1	Phase 2	Phase 3
Corn	77.85	80.55	83.85
Soybean meal, (46.5% CP)	19.70	17.25	13.95
Monocalcium P, (21% P)	0.40	0.30	0.30
Limestone	1.00	1.00	1.00
Sodium chloride	0.35	0.35	0.35
L-Lys-HCl	0.30	0.25	0.23
DL-Met	0.06	0.02	0.01
L-Thr	0.08	0.06	0.06
L-Trp	0.02	0.01	0.01
Trace mineral premix	0.10	0.10	0.10
Vitamin premix	0.10	0.10	0.10
HiPhos 2700	0.02	0.02	0.02
Chromium propionate <sup>1</sup>	---	---	---
Total	100.00	100.00	100.00
Calculated analysis			
Standardized ileal digestible AA, %			
Lys	0.90	0.80	0.70
Ile:Lys	62	64	65
Leu:Lys	142	153	164
Met:Lys	32	30	31
Met and Cys:Lys	58	58	61
Thr:Lys	62	63	66
Trp:Lys	19	19	19
ME, kcal/lb	1,503	1,506	1,507
Ca, %	0.51	0.48	0.47
P, %	0.43	0.40	0.38
Available P, %	0.25	0.23	0.22

<sup>1</sup>Chromium propionate, (Kemin Industries, Des Moines, IA) replaced corn in the control diets in each phase at an inclusion rate of 1 lb/ton to provide 200 ppb of chromium.

**Table 2. Effects of chromium supplementation and space allocation on performance and carcass traits of finishing pigs<sup>1,2</sup>**

Item	9.8 ft <sup>2</sup>		6.8 ft <sup>2</sup>		SEM	Probability, <i>P</i> <		
	Control	Chromium <sup>3</sup>	Control	Chromium <sup>3</sup>		Diet × space	Diet	Space
d 0 to 14								
ADG, lb	2.16	2.09	1.96	1.96	0.042	0.535	0.498	0.007
ADFI, lb	5.30	5.17	4.85	5.02	0.078	0.109	0.825	0.002
F/G	2.46	2.48	2.48	2.57	0.029	0.419	0.218	0.201
d 14 to 28								
ADG, lb	2.21	2.26	2.04	2.04	0.028	0.488	0.505	0.001
ADFI, lb	6.27	6.53	5.86	5.87	0.081	0.173	0.151	0.001
F/G	2.84	2.89	2.88	2.87	0.052	0.654	0.737	0.848
d 28 to 42								
ADG, lb	2.22	2.32	2.10	1.96	0.047	0.014	0.683	0.001
ADFI, lb	6.74	6.75	6.26	6.10	0.076	0.396	0.457	0.001
F/G	3.04	2.91	3.00	3.12	0.049	0.066	0.911	0.205
d 42 to 56								
ADG, lb	2.33	2.21	2.04	2.01	0.032	0.287	0.115	0.001
ADFI, lb	7.04	7.07	6.28	6.28	0.056	0.863	0.850	0.001
F/G	3.02	3.21	3.10	3.12	0.047	0.190	0.123	0.943
d 56 to 72								
ADG, lb	2.24	2.17	2.09	1.98	0.041	0.593	0.044	0.001
ADFI, lb	6.94	7.04	6.28	6.02	0.082	0.033	0.305	0.001
F/G	3.11	3.25	3.01	3.06	0.050	0.527	0.204	0.056
d 0 to 72								
ADG, lb	2.23	2.21	2.05	1.99	0.016	0.410	0.079	0.001
ADFI, lb	6.46	6.52	5.91	5.86	0.042	0.385	0.991	0.001
F/G	2.90	2.95	2.89	2.95	0.021	0.931	0.021	0.689
F/G <sub>adj</sub> <sup>4</sup>	2.82	2.88	2.88	2.96	0.021	0.826	0.014	0.020
BW, lb								
d 0	128.6	128.6	128.6	128.6	1.43	1.000	0.881	0.940
d 14	158.8	157.8	156.2	156.0	1.82	0.607	0.476	0.013
d 28	191.0	189.7	184.8	184.6	1.62	0.637	0.531	0.001
d 42	222.0	222.2	214.2	212.1	1.49	0.412	0.468	0.001
d 56	254.6	253.0	242.7	240.3	1.49	0.795	0.235	0.001
d 72	290.5	287.8	276.1	271.8	1.50	0.649	0.056	0.001

*continued*

**Table 2. Effects of chromium supplementation and space allocation on performance and carcass traits of finishing pigs<sup>1,2</sup>**

Item	9.8 ft <sup>2</sup>		6.8 ft <sup>2</sup>		SEM	Probability, <i>P</i> <		
	Control	Chromium <sup>3</sup>	Control	Chromium <sup>3</sup>		Diet × space	Diet	Space
HCW, lb	213.7	213.2	205.3	201.2	1.18	0.231	0.126	0.001
Yield, %	72.08	72.38	72.69	72.48	0.121	0.054	0.728	0.009
BF, mm	22.05	23.39	20.14	20.53	0.324	0.301	0.069	0.003
LD, mm	55.44	56.26	55.31	54.52	0.538	0.301	0.983	0.441
Lean, %	50.92	50.55	51.54	51.25	0.164	0.849	0.131	0.060

<sup>1</sup> A total of 256 finishing pigs (DNA line 600 × 241, initially 128.6 lb) were used in a 72-d study.

<sup>2</sup> Each pen contained 8 pigs, and different space allocations (9.8 and 6.8 ft<sup>2</sup>) were obtained by adjusting gates.

<sup>3</sup> Chromium propionate 0.04% (Kemin Industries, Des Moines, IA) replaced corn in the control diets in each phase to obtain the chromium diets (1 lb/ton to provide 200 ppb of chromium).

<sup>4</sup>  $F/G_{adj} = (274 - BW \text{ on d } 72) \times 0.005 + \text{actual } F/G$  to adjust to a common ending BW.

## Effects of Increasing Space Allowance by Removing a Pig or Gate Adjustment on Finishing Pig Growth Performance

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### Summary

A total of 256 pigs (PIC 327 × 1050; initially 123.1 lb) were used in a 71 d growth study to compare the effects of increasing space allowance by removing a pig or gate adjustment, on finishing pig growth performance. At the initiation of the trial, pens of pigs were blocked by BW and allotted to 1 of 4 space allowance treatments. The 4 treatments included: 1) 9.8 ft<sup>2</sup>/pig or 2) 6.8 ft<sup>2</sup>/pig for the entire study with treatments 3 and 4 initially providing 6.8 ft<sup>2</sup>, but either a gate was adjusted or the heaviest pig in the pen was removed to provide more space. By using the following equation, space adjustments were made to keep the pigs above their predicted minimum space requirement before growth is impacted: space [(m<sup>2</sup>) = 0.0336 × BW (kg)<sup>0.66</sup>]. There were initially 8 pigs per pen and 8 pens per treatment.

From d 0 to 28, before any gate adjustments or pig removals, ADG tended to be greater ( $P = 0.076$ ) for pigs allowed 9.8 ft<sup>2</sup> compared with pigs stocked at 6.8 ft<sup>2</sup>. Overall, d 0 to 71, pigs allowed 9.8 ft<sup>2</sup> had greater ( $P = 0.001$ ) ADG compared with pigs with all other space allowances. Removing pigs or adjusting the gating increased ( $P = 0.001$ ) ADG compared to those maintained at 6.8 ft<sup>2</sup>; however, both treatments had decreased ( $P = 0.001$ ) ADG compared with pigs allowed 9.8 ft<sup>2</sup>. Most of the differences in ADG can be explained by differences in ADFI. Pigs allowed 9.8 ft<sup>2</sup> had greater ( $P = 0.001$ ) ADFI compared with pigs allowed 6.8 ft<sup>2</sup>; however, intake was similar for pigs allowed increased space by gate adjustment to pigs allowed 9.8 ft<sup>2</sup>. Pigs allowed increased space by pig removal had similar ADFI to pigs allowed 6.8 ft<sup>2</sup>. Space allowance did not influence feed efficiency.

In summary, as expected, pigs with 9.8 ft<sup>2</sup> grew faster and consumed more feed than pigs that were restricted in space. Furthermore, either removing a pig or adjusting the gating as pigs reached the critical  $k$  value influenced growth performance similarly. We speculated that along with pig growth, removing the heaviest pigs could have influenced social dynamics of the remaining pigs in the pen; however, our study indicates the performance benefit from removing the heaviest pig from the pen is primarily from

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the increased space allowance alone. As pigs grew to the minimum predicted space requirement and were subsequently allowed more space, performance was not similar compared to unrestricted pigs. This indicates the industry accepted minimum space prediction equation [ $m^2 = 0.0336 \times BW \text{ (kg)}^{0.66}$ ] doesn't fully explain the impacts on pig performance across multiple body weight ranges.

Key words: space allowance, K-value, marketing

## Introduction

Facility space is the second largest cost of pig production and efficiently using the space is important to maintain profitable pork production. A common allometric expression has been used to describe the relationship between floor space and pig BW, similar to that used to describe volume and surface area. Gonyou et al. (2006)<sup>2</sup> used the allometric expression  $A = k \cdot BW^{0.66}$ , where  $A$  is area allowed per pig ( $m^2$ ),  $k$  is a coefficient, and  $BW$  is pig weight (kg), which converts  $BW$  into a 2-dimensional concept, to describe floor space allowance in order to predict productivity. Using this  $k$  value, 0.0336, the equation should indicate when crowding begins to limit growth. Pig growth should not be decreased until their  $BW$  reaches the point where there is inadequate space to maintain maximal growth rate, (i.e., a  $k$  coefficient less than the critical  $k$  value; Gonyou et al., 2006).

A study by Flohr et al. (2015)<sup>3</sup> suggested reductions in growth due to inadequate space allowance may start to occur before pigs reach the critical  $k$  value. They also reported that removing pigs before the entire pen is marketed increases space allowance for remaining pigs in the pen and increases pig growth performance (Flohr et al., 2015). However, it has not been evaluated whether the improvements in growth are due to the change in social dynamic from removing the heaviest pig, or simply the increased space in the pen as a result of removing the heaviest pig. Thus, the objective of our study was to determine whether the increase in growth rate that occurs when pigs are removed from pens during marketing is due to increasing space allowance by pig removal or gate adjustment during the finishing period.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility was totally enclosed and environmentally controlled, containing 32 pens. Pens were  $8 \times 10$  ft, equipped with adjustable gates to allow different space allowances per pig, completely slatted floors and deep pits for manure storage. Each pen was equipped with a dry single-sided feeder (Farmweld, Teutopolis, IL) with two  $14 \times 10$  in (width  $\times$  depth)

<sup>2</sup> Gonyou, H. W., M. C. Brumm, E. Bush, J. Deen, S. A. Edwards, R. Fangman, J. J. McGlone, M. Meunier-Salaun, R. B. Morrison, H. Spooler, P. L. Sundberg, and A. K. Johnson. 2006. Application of broken-line analysis to assess floor space requirements of nursery and grower-finisher pigs expressed on an allometric basis. *J. Anim. Sci.* 84:229–235.

<sup>3</sup> Flohr, J. R.; Tokach, M. D.; Patience, John F.; Gourley, G.; DeRouchey, J. M.; Dritz, S. S.; Woodworth, J. C.; and Goodband, R. D. (2015). "Re-evaluating floor space allowance and removal strategy effects on the growth of heavyweight finishing pigs." *Kansas Agricultural Experiment Station Research Reports*: Vol. 1: Iss. 7.

feeder spaces and a 1-cup waterer, which provided ad libitum access to feed and water. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen.

A total of 256 pigs (PIC 327 × 1050; initially 123.1 lb) were used in a 71 d growth study. Pens of pigs were blocked by BW and allotted to 1 of 4 space allowance treatments, initially with 8 pigs per pen (4 barrows and 4 gilts) and 8 pens per treatment. The 4 treatments included pens with 9.8 ft<sup>2</sup>/pig or 6.8 ft<sup>2</sup>/pig for the entire study. Two additional treatments initially provided 6.8 ft<sup>2</sup>, but either a gate was adjusted on d 28, 45, and 62 or the heaviest pig in the pen was removed from the pen on d 28 and 45 to provide more space (Table 1). The space adjustments and pig removals were made to keep the pigs above their predicted minimum space requirement [(m<sup>2</sup>) = 0.0336 × BW (kg)<sup>0.66</sup>], where 0.0336 is the *k* value. If a pig died or was removed from a pen during the experiment, pen size was adjusted to maintain the correct space allowance per pig.

Pigs were fed a common corn-soybean-meal based diet offered in 3 phases (Table 2). Diets were formulated to meet or exceed the pigs' nutrient requirement estimates (NRC, 2012)<sup>4</sup> and the 3 phases were fed from approximately 123 to 185, 185 to 220, and 220 to 280 lb BW. Diets were sampled and subsamples were sent to a commercial laboratory (Ward Laboratories Inc., Kearney, NE) for analysis (DM and CP; Table 2). Pens of pigs and feeders were weighed on d 0, 14, 28, 45, 62, and 71 to calculate ADG, ADFI, and F/G.

Data were analyzed as a generalized randomized block design with space allowance treatment as a fixed effect and block as a random effect using PROC MIXED in SAS (SAS Institute, Inc., Cary, NC), with pen serving as the experimental unit. Treatment means were separated using the DIFFS option from the LSMEANS statement of SAS. Results were considered significant at  $P \leq 0.05$  and a tendency at  $P > 0.05$  and  $P \leq 0.10$ .

## Results and Discussion

From d 0 to 14, there was no effect of stocking density observed for ADG, ADFI, and F/G, which corresponded to a change in BW from approximately 123 to 153 lb (Table 3). From d 14 to 28, pigs provided 9.8 ft<sup>2</sup> had increased ADFI ( $P = 0.041$ ) and ADG ( $P = 0.002$ ), which resulted in improved F/G ( $P = 0.025$ ) and a tendency for increased ( $P = 0.081$ ) BW, compared to pigs provided 6.8 ft<sup>2</sup>. These observations suggest space restriction started to influence growth rate between 153 and 182 lb BW. Based on a *k* value of 0.0336, no differences in pig performance were expected before d 28 which corresponded to BW of approximately 182 lb. From d 0 to 28, before any gate adjustments or pig removals, ADG tended to be greater ( $P = 0.076$ ) for pigs allowed 9.8 ft<sup>2</sup> compared to pigs stocked at 6.8 ft<sup>2</sup> for the duration of the study.

From d 28 to 45, pigs provided 6.8 ft<sup>2</sup> or increasing space allowance by removal of the heaviest pig, had decreased ( $P = 0.025$ ) ADFI compared to pigs provided 9.8 ft<sup>2</sup> with pigs from pens where the gate was adjusted being intermediate. This suggests when the heaviest pig is removed from a pen, pigs did not maintain feed intake similar to pigs allowed 9.8 ft<sup>2</sup>.

<sup>4</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, D.C.

From d 45 to 62, ADFI was decreased ( $P = 0.001$ ) for pigs provided 6.8 ft<sup>2</sup> compared to all other treatments. During this period, increasing space allowance resulted in performance similar to pigs allowed 9.8 ft<sup>2</sup>. On d 62, gates were adjusted to reach the desired  $k$  value; however, a pig was not removed from the pig removal treatment because the critical  $k$  value was reached sooner based on the actual ft<sup>2</sup> for pigs in the gate adjustment treatment than for the pig removal treatment.

From d 62 to 71, ADG decreased ( $P = 0.008$ ) when pigs were allowed 6.8 ft<sup>2</sup> compared to all other treatments, which is likely due to the decreased ( $P = 0.001$ ) ADFI, because F/G was not affected.

For the cumulative period after space adjustments began (d 28 to 71), both ADG and ADFI decreased ( $P = 0.001$ ) when pigs were provided 6.8 ft<sup>2</sup> compared with pigs provided 9.8 ft<sup>2</sup>. Pigs provided increased space by removing pigs had similar performance to those where gates were adjusted to increase space; however, pig removal resulted in lower ADG and ADFI than pigs allowed 9.8 ft<sup>2</sup> throughout the experiment.

Overall (d 0 to 71), pigs provided 9.8 ft<sup>2</sup> had increased ( $P = 0.001$ ) ADG compared with all other treatments. Performance of pigs with gate adjustment or pig removal was similar, and both having greater ADG than pigs provided 6.8 ft<sup>2</sup>. Pigs provided 9.8 ft<sup>2</sup> had increased ( $P = 0.001$ ) ADFI compared with pigs allowed 6.8 ft<sup>2</sup>; however, intake was similar among pigs provided increased space by gate adjustment to pigs allowed 9.8 ft<sup>2</sup>. Pigs provided increased space by pig removal had similar ADFI to pigs allowed 6.8 ft<sup>2</sup>. Final BW was decreased ( $P = 0.001$ ) for pigs provided 6.8 ft<sup>2</sup> compared with those provided 9.8 ft<sup>2</sup>. Also, final BW of pigs provided increased space by adjusting the gate was greater ( $P = 0.001$ ) than pigs allowed 6.8 ft<sup>2</sup> or increased space by pig removal, but decreased ( $P = 0.001$ ) compared to pigs provided 9.8 ft<sup>2</sup>.

Gonyou et al. (2006) reported that ADFI was decreased when pigs were stocked below a critical  $k$  value of 0.0336, which is also supported by our study. Reductions in performance have been observed due to inadequate space allowance, which may start to occur before the pigs reach their critical  $k$  value. This is a similar key finding in our study where pig performance was reduced before the critical  $k$  value was reached, which confirms recent research of Flohr et al. (2015). Furthermore, our data suggest improved growth performance after pigs are removed during the finishing period may be largely due to the increased space provided to pigs remaining in the pens because performance was similar to that of pigs where space was increased by adjusting the gate (without removing the heaviest pig).

In this study, pigs with greater space allowance grew faster and consumed more feed than pigs that were restricted in space. Furthermore, either removing a pig or adjusting the gating as pigs reached the critical  $k$  value influenced growth performance similarly. We speculated that along with pig growth, social dynamics of the remaining pigs in the pen could have been influenced by removing the heaviest pigs; however, our study indicates the performance benefit from removing the heaviest pig from the pen is primarily from the increased space allowance alone. Lastly, as pigs grew to the minimum predicted space requirement and were subsequently provided more space, performance was not similar to unrestricted pigs. Increasing the space allowance by removing pigs

or adjusting the gating increased ADG compared to pigs provided 6.8 ft<sup>2</sup> for the entire experiment; however, neither treatment allowed pigs to maintain ADG similar to pigs provided 9.8 ft<sup>2</sup> throughout the study. This indicates the industry accepted minimum space prediction equation [(m<sup>2</sup>) = 0.0336 × BW (kg)<sup>0.66</sup>] doesn't fully explain impacts on pig performance across multiple body weight ranges.

**Table 1. Space allowance and *k* value through the experiment<sup>1</sup>**

Item	9.8 ft <sup>2</sup>	6.8 ft <sup>2</sup>	Gate adjustment <sup>2</sup>	Pig removal <sup>3</sup>
d 0				
ft <sup>2</sup> /pig <sup>4</sup>	9.8	6.8	6.8	6.8
<i>k</i> value <sup>5</sup>	0.0615	0.0427	0.0427	0.0427
d 28				
ft <sup>2</sup> /pig	9.8	6.8	7.8	7.8
<i>k</i> value				
Before adj.	0.0473	0.0328	0.0377	0.0377
After adj.	---	---	0.0425	0.0439
d 45				
ft <sup>2</sup> /pig	9.8	6.8	8.8	9.1
<i>k</i> value				
Before adj.	0.0422	0.0293	0.0379	0.0392
After adj.	---	---	0.0422	0.0392
d 62				
ft <sup>2</sup> /pig	9.8	6.8	9.8	9.1
<i>k</i> value				
Before adj.	0.0368	0.0255	0.0368	0.0342
After adj.	---	---	0.0368	0.0409
d 71				
ft <sup>2</sup> /pig	9.8	6.8	9.8	9.1
<i>k</i> value	0.0361	0.0250	0.0361	0.0335

<sup>1</sup>A total of 256 pigs (PIC 327 × 1050, initially 123.1 lb) were used in a 71 d growth trial. Average BW on d 0, 28, 45, 62 and 71 was 123, 182, 216, 265, and 273 lb, respectively.

<sup>2</sup>Increased space by gate adjustment (d 28, 45, and 62).

<sup>3</sup>Increased space by heaviest pig removal (d 28 and 45).

<sup>4</sup>Indicates area maintained (ft<sup>2</sup>/pig) between each data collection period.

<sup>5</sup>*k*-value [(m<sup>2</sup>) = *k* × BW (kg)<sup>0.66</sup>] calculated before and after a pig was removed or gates were adjusted.

**Table 2. Diet composition (as-fed basis)**

Ingredient, %	Phase <sup>1</sup>		
	1	2	3
Corn	71.50	78.44	82.86
Soybean meal, 47.7% CP	25.71	19.20	14.93
Monocalcium P, 21% P	0.55	0.33	0.30
Limestone	1.13	1.10	1.08
Salt	0.35	0.35	0.35
L-Lys HCl	0.31	0.25	0.22
DL-Met	0.06	0.02	---
L-Thr	0.09	0.05	0.05
Trace mineral premix	0.15	0.13	0.10
Vitamin premix	0.15	0.13	0.10
Phytase <sup>2</sup>	0.02	0.02	0.02
Total	100.00	100.00	100.00
Calculated analysis			
Standardized ileal digestible (SID) AA's, %			
Lys	1.05	0.85	0.72
Ile:Lys	62	64	66
Met:Lys	30	29	30
Met and Cys:Lys	55	56	59
Thr:Lys	61	61	64
Trp:Lys	18.0	18.0	18.0
Val:Lys	69	73	76
Total Lys, %	1.18	0.96	0.82
ME, kcal/lb	1,494	1,501	1,504
NE, kcal/lb	1,117	1,137	1,150
SID Lys:ME, g/Mcal	3.19	2.57	2.17
CP, %	18.5	15.9	14.2
Ca, %	0.62	0.55	0.52
P, %	0.49	0.41	0.39
Available P, %	0.29	0.23	0.22
Chemical analysis <sup>3</sup> , %			
DM	88.32	87.25	87.41
CP	18.5	15.4	14.8

<sup>1</sup>Phases 1, 2, and 3 were fed d 0 to 28, 28 to 45, and 45 to 71, respectively.

<sup>2</sup>HiPhos (DSM Inc, Parsippany, NJ) provided 1,228,503 (FYT)/lb of product and released 0.10% available P.

<sup>3</sup>Multiple samples of each diet were collected, blended and subsampled, and analyzed (Ward Laboratories, Inc. Kearney, NE). Values are represented on an as fed basis.

**Table 3. Effects of pig space allowance on finishing pig growth performance<sup>1,2</sup>**

Item	9.8 ft <sup>2</sup>	6.8 ft <sup>2</sup>	Gate adjustment <sup>3</sup>	Pig removal <sup>4</sup>	SEM	<i>P</i> <
<b>BW, lb</b>						
d 0	123.3	123.4	123.2	122.6	0.33	0.361
d 14	152.3	152.6	153.0	152.3	0.57	0.835
d 28	185.3 <sup>x</sup>	181.5 <sup>y</sup>	182.1 <sup>y</sup>	182.6 <sup>y</sup>	1.03	0.081
d 45	221.8 <sup>a</sup>	214.5 <sup>b</sup>	216.5 <sup>b</sup>	214.8 <sup>b</sup>	1.07	0.001
d 62	261.4 <sup>a</sup>	252.5 <sup>c</sup>	256.8 <sup>b</sup>	251.4 <sup>c</sup>	1.39	0.001
d 71	280.6 <sup>a</sup>	268.3 <sup>c</sup>	275.4 <sup>b</sup>	270.0 <sup>c</sup>	1.60	0.001
<b>d 0 to 14</b>						
ADG, lb	2.07	2.08	2.13	2.13	0.032	0.495
ADFI, lb	4.83	4.75	4.83	4.84	0.097	0.894
F/G	2.33	2.28	2.27	2.28	0.045	0.752
<b>d 14 to 28</b>						
ADG, lb	2.32 <sup>a</sup>	2.06 <sup>b</sup>	2.08 <sup>b</sup>	2.15 <sup>b</sup>	0.045	0.002
ADFI, lb	5.70 <sup>a</sup>	5.32 <sup>b</sup>	5.51 <sup>b</sup>	5.60 <sup>b</sup>	0.091	0.041
F/G	2.45 <sup>a</sup>	2.57 <sup>b</sup>	2.65 <sup>b</sup>	2.60 <sup>b</sup>	0.047	0.025
<b>d 0 to 28</b>						
ADG, lb	2.19 <sup>x</sup>	2.07 <sup>y</sup>	2.10 <sup>xy</sup>	2.14 <sup>xy</sup>	0.033	0.076
ADFI, lb	5.27	5.03	5.17	5.22	0.078	0.200
F/G	2.40	2.43	2.46	2.44	0.033	0.541
<b>d 28 to 45</b>						
ADG, lb	2.15	1.94	2.02	2.06	0.061	0.143
ADFI, lb	6.32 <sup>a</sup>	5.93 <sup>b</sup>	6.14 <sup>ab</sup>	5.90 <sup>b</sup>	0.102	0.025
F/G	2.96	3.08	3.05	2.87	0.080	0.240
<b>d 45 to 62</b>						
ADG, lb	2.33	2.24	2.38	2.32	0.049	0.260
ADFI, lb	7.06 <sup>a</sup>	6.40 <sup>b</sup>	6.96 <sup>a</sup>	6.87 <sup>a</sup>	0.100	0.001
F/G	3.03	2.87	2.94	2.98	0.056	0.237
<b>d 62 to 71</b>						
ADG, lb	2.12 <sup>a</sup>	1.75 <sup>b</sup>	2.06 <sup>a</sup>	2.06 <sup>a</sup>	0.077	0.008
ADFI, lb	6.43 <sup>a</sup>	5.88 <sup>b</sup>	6.56 <sup>a</sup>	6.46 <sup>a</sup>	0.101	0.001
F/G	3.07	3.39	3.20	3.16	0.127	0.334

*continued*

**Table 3. Effects of pig space allowance on finishing pig growth performance<sup>1,2</sup>**

Item	9.8 ft <sup>2</sup>	6.8 ft <sup>2</sup>	Gate adjustment <sup>3</sup>	Pig removal <sup>4</sup>	SEM	<i>P</i> <
d 28 to 71						
ADG, lb	2.21 <sup>a</sup>	2.01 <sup>c</sup>	2.17 <sup>ab</sup>	2.15 <sup>b</sup>	0.029	0.001
ADFI, lb	6.64 <sup>a</sup>	6.10 <sup>c</sup>	6.55 <sup>ab</sup>	6.37 <sup>b</sup>	0.077	0.001
F/G	3.00	3.03	3.02	2.96	0.033	0.441
d 0 to 71						
ADG, lb	2.21 <sup>a</sup>	2.04 <sup>c</sup>	2.14 <sup>b</sup>	2.15 <sup>b</sup>	0.021	0.001
ADFI, lb	6.09 <sup>a</sup>	5.68 <sup>c</sup>	6.01 <sup>ab</sup>	5.85 <sup>bc</sup>	0.063	0.001
F/G	2.76	2.81	2.82	2.77	0.030	0.486

<sup>1</sup>A total of 256 pigs (PIC 327 × 1050; initially 123.1 lb) were used in a 71 d growth trial with 8 replications/treatment to determine the effects of space allowance on finishing pig growth performance.

<sup>2</sup>Means within a row with different superscripts differ: <sup>abc</sup> *P* < 0.05, <sup>xyz</sup> *P* < 0.10

<sup>3</sup>Increased space = increased gate adjustment; initially 6.8 ft<sup>2</sup>/ pig with gates adjusted as pigs reached the *k* value, to be non-limiting (7.8 ft<sup>2</sup> at 180 lb (d 28), 8.8 ft<sup>2</sup> at 220 lb (d 45), and 9.8 ft<sup>2</sup> at 260 lb (d 62)).

<sup>4</sup>Increased space = removal of heaviest pig; initially 6.8 ft<sup>2</sup>/ pig with a pig removed as the *k* value is reached to be non-limiting: 1 pig at 180 lb (d 28) and 220 lb (d 45).

## Effects of Increasing Zn from Zinc Sulfate or Zinc Hydroxychloride on Finishing Pig Growth Performance, Carcass Characteristics, and Economic Return<sup>1</sup>

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### Summary

A total of 1,008 pigs [TR4 (Fast × L02 PIC; initially 70.6 lb BW)] were used in a 103-d growth study to determine the effects of Zn source and level on finishing pig growth performance, carcass characteristics, and economic return. The 6 dietary treatments were arranged as a 2 × 3 factorial with main effects of Zn source (ZnSO<sub>4</sub>; Agrium Advance Technology, Loveland, CO, or Zn hydroxychloride; Intellibond-Z; Micronutrients, Indianapolis, IN) and level (50, 100, or 150 ppm added Zn). The trace mineral premix was formulated to contain no added Zn. There were 21 pigs per pen and 8 pens per treatment.

Overall, there was no effect of Zn source for growth performance criteria observed. Increasing added Zn maximized (quadratic,  $P = 0.007$ ) ADG when diets contained 100 ppm Zn; however, F/G tended to worsen (source × level, linear,  $P = 0.068$ ) as Zn from Zn hydroxychloride increased, but was relatively unchanged when pigs were fed increasing Zn from ZnSO<sub>4</sub>. Carcass yield increased (linear,  $P = 0.027$ ) as Zn level increased. Pigs fed diets with Zn hydroxychloride had heavier ( $P = 0.041$ ) HCW, and increased HCW ADG ( $P = 0.036$ ) than those fed ZnSO<sub>4</sub>. Hot carcass weight and HCW ADG were maximized (quadratic,  $P \leq 0.006$ ) when diets contained 100 ppm Zn. There was a tendency for income over feed cost (IOFC) to be maximized when pigs were fed diets with 100 ppm Zn when economic analysis was calculated on both a constant day (quadratic,  $P = 0.059$ ) and constant carcass weight (quadratic,  $P = 0.070$ ) basis, respectively.

<sup>1</sup> Appreciation is expressed to New Fashion Pork, Worthington, MN, for use of feed mill and research facilities, and to Chad Hastad and Ryan Cain for technical assistance. The authors would also like to express appreciation to Micronutrients, Inc, Indianapolis, IN, for partial funding.

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<sup>4</sup> Micronutrients, Inc, Indianapolis, IN

In summary, these results suggest that a total of 100 ppm added Zn is adequate to maximize ADG, HCW, HCW ADG, and IOFC, but F/G worsened as Zn level increased. Zinc source did not affect growth performance; however, pigs fed Zn hydroxychloride had increased HCW and HCW ADG compared to those fed ZnSO<sub>4</sub>.

Key words: finishing pig, zinc hydroxychloride, zinc sulfate

## Introduction

Historically, increasing Zn above that provided from the trace mineral premix [generally around 50 ppm (NRC, 2012)<sup>5</sup>] has not been added in finishing pig diets. However, some recent research suggests improvements in growth performance with increasing levels of added Zn (75 ppm), especially during the finisher period when ractopamine HCl is fed (Paulk et al., 2014)<sup>6</sup>. These studies suggest that further research is necessary to re-examine the Zn requirement of grow-finish pigs. Furthermore, while some nursery pig data are available to compare Zn sources, no data are available to compare the effects of Zn hydroxychloride, a unique form of inorganic Zn, to other more commonly used forms of Zn (ZnSO<sub>4</sub>) in the finisher phase. Therefore, our study was designed to investigate the effects of increasing Zn from two different sources on growth performance, carcass characteristics, and economic return of finishing pigs housed in a commercial environment.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. This study was conducted at New Fashion Pork in a commercial research facility in Round Lake, MN. The research barn was double-curtain-sided with completely slatted flooring and deep pits for manure storage. Pigs had approximately 7.4 ft<sup>2</sup>/pig and each pen was equipped with a 5-hole stainless steel dry self-feeder (Thorp Equipment, Inc., Thorp, WI) and a cup waterer for ad libitum access to feed and water. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN). Research diets were manufactured in a commercial feed mill located in Estherville, IA.

A total of 1,008 pigs (TR4 (Fast × L02 PIC); initially 70.6 lb BW) were used in a 103-d growth experiment to determine the effects of increasing Zn from two different sources on finishing pig growth performance, carcass characteristics, and economic return. Pigs were allotted to pen based on initial body weight with 8 pens per treatment and 21 pigs per pen (mixed gender) and pens were randomly allotted to 1 of the 6 dietary treatments. The 6 dietary treatments were arranged as a 2 × 3 factorial with main effects of Zn source (ZnSO<sub>4</sub> or Zn hydroxychloride; Intellibond Z; Micronutrients, Indianapolis, IN) and Zn level (50, 100, or 150 ppm). All diets were corn- soybean meal-DDGS based and were fed in 5 phases (approximately 70 to 100, 100 to 140, 140 to 180, 180 to 230, and 230 to 280 lb) with ractopamine HCl included in the final phase (Table 1). The trace mineral premix added to all diets contained no added Zn.

<sup>5</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, D.C.

<sup>6</sup> Paulk et al., Swine Day 2014, Report of Progress 1110, pp. 164-171. Kansas Agricultural Experiment Station, Manhattan, KS.

Complete diet samples were collected from a minimum of 6 feeders per phase and combined to make 1 composite sample per treatment and phase. Each sample was split and ground then sent to Cumberland Valley Analytical Services (Hagerstown, MD) and Ward Laboratories Inc. (Kearney, NE) for analysis of DM, CP, ADF, crude fiber, Ca, P, ether extract, ash, starch and Zn concentrations. Final Zn concentrations were determined by averaging a total of 3 values; 1 analyzed value from Ward Laboratories Inc. and 2 analyzed values from Cumberland Valley Analytical Services.

Pigs were weighed and feed disappearance was measured approximately every 2 weeks to calculate ADG, ADFI, and F/G. On d 89 of the trial, pens were weighed and the 6 heaviest pigs from each pen were removed and transported 350 miles to Triumph Foods (St. Joseph, MO) for harvest. The remaining pigs were transported to Triumph Foods on d 103 for harvest. Carcass yield was calculated using HCW at the plant divided by live weight at the farm on an individual pig basis. Standard carcass measurements of backfat and loin depth were measured with pen as experimental unit and carcass as the observational unit. Percentage lean was calculated using equations from the National Pork Producers Council (2000). Hot carcass weight ADG was calculated by subtracting initial HCW from the final HCW obtained at the plant, then divided by 103 d on test. An assumed carcass yield of 75% was used to calculate initial HCW at the beginning of the experiment. Hot carcass weight F/G was calculated by dividing the pen total feed intake divided by pen total carcass weight gain.

Economical comparisons were made based on both a constant ending weight and a constant day basis. For both, total feed cost per pig, cost per pound of gain, carcass ADG and F/G, value and income over feed cost (IOFC) were calculated. Feed cost was calculated by multiplying total feed intake per pig by a weighted mean diet cost on a per pen basis. Prices used for corn, soybean meal, and DDGS at the time of the experiment were \$0.05, 0.14, and 0.04/lb, respectively. Prices used for the Zn hydroxychloride and ZnSO<sub>4</sub> were \$2.80 and 0.69/lb, respectively. Carcass price at time of slaughter was calculated at \$0.82 per pound. Cost per pound of gain was calculated by dividing the total feed cost per pig by the total carcass pounds gained overall. The value of the carcass weight gained during the experiment (gain value) was calculated by multiplying the carcass value by the product of the pen final carcass weight yield. Income over feed cost was calculated by subtracting total feed cost from gain value. The income over feed and facilities cost (IOFFC) was calculated for the constant market weight evaluation because pigs with faster growth rates will reach a 210 lb carcass sooner, therefore decreasing housing costs. Facility cost was calculated by multiplying the number of overall days the pigs need to reach a 210 lb carcass based on their respective growth rate by \$0.10 per head per day facility cost.

Data were analyzed as a randomized complete block design using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Hot carcass weight was used as a covariate for carcass characteristics including percentage lean, loin depth, and backfat. Both linear and quadratic effects of source and level were analyzed with significance defined as  $P < 0.05$  and a tendency as  $P < 0.10$  and  $\geq 0.05$ .

## Results and Discussion

The chemical analyses of the complete diets were similar to the intended formulation (Table 2, 3, and 4). Total Ca and P concentrations were similar among diets across each dietary phase. The total analyzed Zn concentrations for diets formulated to 50, 100, and 150 ppm added zinc from ZnSO<sub>4</sub> ranged from; 83 to 202, 150 to 200, and 183 to 225 ppm, respectively. Total analyzed Zn levels for diets formulated to 50, 100, and 150 ppm added zinc from Zn hydroxychloride ranged from; 101 to 121, 128 to 176, and 178 to 226 ppm, respectively.

From d 0 to 33, neither Zn source nor level influenced growth performance. From d 33 to 66, there were no Zn source × level interactions for ADG or ADFI; however, F/G worsened when 150 ppm of Zn from ZnSO<sub>4</sub> was added, whereas, poorer F/G was first observed when 100 ppm of Zn from Zn hydroxychloride was added (source × level, quadratic,  $P = 0.007$ ; Table 5). There was a tendency for ADG to increase then decrease (quadratic,  $P = 0.092$ ) and ADFI increased (linear,  $P = 0.042$ ) with increasing Zn. This resulted in poorer (linear,  $P = 0.001$ ) F/G with increasing added Zn. Pigs fed ZnSO<sub>4</sub> tended to have better F/G ( $P = 0.096$ ) compared with those fed Zn hydroxychloride.

From d 66 to 103, there were no Zn source × level interactions observed for ADG or ADFI; however, as Zn from Zn hydroxychloride increased, F/G became poorer (source × level, linear,  $P = 0.007$ ). Increasing Zn increased ADG (quadratic,  $P = 0.001$ ) and tended to increase ADFI (quadratic,  $P = 0.051$ ) through 100 ppm, but when 150 ppm was included performance returned to levels similar to those fed 50 ppm. Pigs fed Zn from Zn hydroxychloride had greater ADFI ( $P = 0.026$ ) than those fed ZnSO<sub>4</sub>. Feed efficiency improved (quadratic,  $P = 0.011$ ) and was maximized when pigs were fed 100 ppm of Zn compared with those fed 50 or 150 ppm which had similar F/G.

Overall, (d 0 to 103), there were no Zn source × level interactions observed for ADG or ADFI; however, F/G tended to worsen (source × level, linear,  $P = 0.068$ ) as Zn from Zn hydroxychloride increased, but was relatively unchanged when pigs were fed increasing Zn from ZnSO<sub>4</sub>. Final BW and ADG were maximized (quadratic,  $P \leq 0.011$ ) when pigs were fed 100 ppm of Zn. Carcass yield increased (linear,  $P = 0.027$ ; Table 6) with increasing added Zn. Pigs fed Zn hydroxychloride had heavier ( $P = 0.041$ ) HCW than those fed added ZnSO<sub>4</sub>. Hot carcass weight increased (quadratic,  $P = 0.006$ ) then decreased and was maximized when diets contained 100 ppm of added Zn. Similarly, pigs fed Zn hydroxychloride had increased ( $P = 0.036$ ) HCW ADG. Hot carcass weight ADG increased (quadratic,  $P = 0.005$ ) then decreased with increasing Zn and was maximized when diets contained 100 ppm of added Zn.

For the economic analysis when reported on a constant time basis, there were no source × level interactions observed for feed cost, carcass gain value or IOFC. However, cost per pound of carcass gain increased (source × level, linear,  $P = 0.002$ ; Table 7) as Zn from Zn hydroxychloride increased, which may be attributed to the poorer (source × level, linear,  $P = 0.005$ ) carcass F/G at the 150 ppm level. Increasing added Zn tended (quadratic,  $P = 0.098$ ) to increase then decrease feed cost and was highest when diets contained 100 ppm of added Zn. Carcass gain value was maximized (quadratic,  $P = 0.011$ ) when pigs were fed 100 ppm of Zn, which resulted in the greatest (quadrat-

ic,  $P = 0.007$ ) IOFC. Because of the improved HCW ADG, carcass gain value increased ( $P = 0.039$ ) for pigs fed Zn hydroxychloride compared with pigs fed  $ZnSO_4$ .

When reported on a constant weight basis, there were no source  $\times$  level interactions observed for facility costs, but a source  $\times$  level interaction ( $P < 0.011$ ) was found for all other response criteria. The interaction occurred because carcass F/G, feed cost, cost/lb of carcass gain, IOFC, and IOFFC were improved for pigs fed 50 or 100 ppm Zn, but poorer for pigs fed 150 ppm Zn from Zn hydroxychloride compared with pigs fed Zn from  $ZnSO_4$ .

It is currently recommended (NRC, 2012) that finishing pigs are fed diets containing 50 ppm of Zn. From our study, it appears that there may be growth promoting benefits to supplementing diets with Zn beyond 50 ppm. The current study suggests 100 ppm of Zn maximizes overall ADG and BW for growing pigs from 70 to 280 lb of BW.

Previous literature suggests there may be performance benefits of added Zn during the earliest stages of finishing, but without any impact on overall growth performance (Paulk et al., 2014)<sup>7</sup>. In their study, the basal diet contained 55 ppm Zn from the trace mineral premix. An addition of 75 ppm of Zn for a total Zn level of 130 ppm did not improve overall performance. These results are not consistent with the findings of the current study which suggest 100 ppm of Zn maximizes overall BW and ADG. Our study also indicates HCW, HCW ADG, and IOFC were maximized when diets contained 100 ppm of Zn. However, Paulk et al. (2014) observed carcass characteristics and economics were not influenced by adding more than 55 ppm of Zn fed to pigs in the early finishing period, late finishing period, or throughout the overall finishing period.

In similar studies that evaluated increasing levels of added Zn from ZnO, a trend for improved feed efficiency was observed (Paulk et al., 2015)<sup>8</sup>. The same authors suggest pigs fed added Zn from ZnO have increased ADG and increased ADFI during the first growth period of their study, compared with those fed added Zn from ZnAA, but with no overall differences in growth performance. In this study, Paulk et al. (2015) used analyzed Zn concentrations ranging from 83 ppm (basal diet) to 267 ppm with the added Zn as ZnAA or ZnO. Although this range of Zn concentration is larger than that of the current study, the ADFI results between the studies are similar. Similar to our study, which suggests differences in ADFI for pigs fed different Zn sources during intermediate growth periods, these differences did not translate into the overall data. Interestingly, our data suggest overall F/G becomes poorer when pigs are fed increasing levels of added Zn; however Paulk et al. (2015) suggests increasing Zn tended to improve feed efficiency. Although the data are mixed on whether or not increasing Zn improves feed efficiency, the studies do agree that F/G is similar when pigs are fed diets containing different Zn sources.

<sup>7</sup> Paulk et al., Swine Day 2014, Report of Progress 1110, pp. 164-171. Kansas Agricultural Experiment Station, Manhattan, KS.

<sup>8</sup> Paulk, C. B., D. D. Burnett, M. D. Tokach, J. L. Nelssen, S. S. Dritz, J. M. DeRouche, R. D. Goodband, G. M. Hill, K. D. Haydon, and J. M. Gonzalez. 2015. Effect of added zinc in diets with ractopamine hydrochloride on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression of finishing pigs. *J. Anim. Sci.* 93:185-196.

In summary, our study suggests little overall differences between Zn sources on growth performance; however, pigs fed diets with Zn hydroxychloride had greater HCW compared to those fed ZnSO<sub>4</sub>. These results suggest 100 ppm of Zn maximizes ADG, HCW, HCW ADG, and IOFC when reported on a constant day or weight basis with a greater response in the later phases (d 66 to 103) of the study. This might suggest that duration of feeding elevated levels of Zn might influence the magnitude of response observed. As a result, more research should be conducted to determine if duration of feeding different levels or sources of Zn influences the magnitude of growth performance response observed.

**Table 1. Diet composition (as-fed basis)**

Item	Phase <sup>1,2</sup>				
	1	2	3	4	5
Ingredient, %					
Corn	48.08	52.13	55.70	58.31	69.00
Soybean meal, 47.5% CP	19.56	15.69	12.24	9.66	18.66
Corn DDGS	30.00	30.00	30.00	30.00	10.00
Monocalcium P, 21% P	0.15	---	---	---	0.30
Limestone	1.35	1.35	1.25	1.25	0.95
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys-HCl	0.35	0.33	0.30	0.28	0.35
L-Thr	---	---	---	---	0.09
L-Trp	0.01	0.01	0.01	---	0.02
Methionine <sup>3</sup>	---	---	---	---	0.10
Ractopamine HCl, 9 g/lb	---	---	---	---	0.03
Vitamin/trace mineral premix <sup>4</sup>	0.15	0.15	0.15	0.15	0.15
Zn source <sup>5</sup>	---	---	---	---	---
Total	100	100	100	100	100

*continued*

**Table 1. Diet composition (as-fed basis)**

Item	Phase <sup>1,2</sup>				
	1	2	3	4	5
Calculated analysis					
Standardized ileal digestible (SID) AA, %					
Lys	0.97	0.86	0.76	0.68	0.9
Ile:lys	71	73	75	77	63
Leu:lys	178	191	207	223	155
Met:lys	29	30	33	35	34
Met + Cys:lys	57	61	65	70	60
Thr:lys	63	65	67	70	65
Trp:lys	18.6	18.7	18.6	18.5	18.6
Val:lys	79	82	86	90	70
Total lys, %	1.11	0.99	0.88	0.8	1
ME, kcal/lb	1,510	1,512	1,514	1,514	1,514
NE, kcal/lb	1,108	1,120	1,118	1,124	1,133
SID Lys:ME, g/Mcal	2.91	2.58	2.28	2.04	2.7
CP, %	20.76	19.18	17.77	16.7	16.73
Ca, %	0.63	0.58	0.53	0.52	0.52
P, %	0.55	0.51	0.49	0.49	0.46
Available P, %	0.41	0.37	0.37	0.36	0.32

<sup>1</sup>Phases 1, 2, 3, 4, and 5 were fed from d 0 to 17, 17 to 33, 33 to 48, 48 to 66, and 66 to 103, respectively.

<sup>2</sup>Dietary treatments were formed by adding 50, 100, 150 ppm of Zn from either ZnSO<sub>4</sub> or Zn hydroxychloride at the expense of corn. All diets were manufactured using a Zn-free trace mineral premix.

<sup>3</sup>MHA, Novus International, Saint Charles, MO.

<sup>4</sup>The vitamin and Zn free trace mineral premix supplied; vitamin A 1,867,000 I.U, vitamin D3 267,000 I.U., vitamin E 12,000 I.U, vitamin B12 7.334 mg, riboflavin (B2) 2,667 mg, niacin 8,000 mg, d-pantothenic acid 5,334 mg, menidione 667 mg, selenium 0.020, copper 10.8, iron 5.07, manganese 1.9. Vitamin concentrations are expressed on a per lb of product basis; whereas mineral concentrations are expressed on a total percentage of premix basis.

<sup>5</sup>ZnSO<sub>4</sub> (Zinc sulfate) (Agrium Advance Technology, Loveland, CO) or Intellibond-Z, Zinc hydroxychloride, (Micronutrients, Indianapolis, IN).

**Table 2. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Phase 1						Phase 2					
	ZnSO <sub>4</sub> <sup>2</sup> , ppm			Zn hydroxychloride <sup>3</sup> , ppm			ZnSO <sub>4</sub> <sup>2</sup> , ppm			Zn hydroxychloride <sup>3</sup> , ppm		
	50	100	150	50	100	150	50	100	150	50	100	150
DM, %	88.10	87.60	88.30	87.60	87.40	87.70	86.60	86.90	86.90	87.10	86.90	86.70
CP, %	22.50	23.00	22.40	21.00	20.90	22.20	20.20	21.60	23.00	21.20	22.20	21.60
Crude fiber, %	4.40	4.60	4.20	4.10	3.90	4.70	3.90	4.30	4.80	4.20	4.30	4.30
Ether extract, %	5.99	5.38	5.54	4.97	4.89	5.22	4.60	4.32	4.51	5.57	4.88	4.18
Ash, %	5.85	5.82	5.87	5.54	5.53	6.21	5.89	6.68	6.64	5.60	6.17	6.25
Ca, %	0.83	0.96	0.98	0.85	0.78	1.02	0.85	0.99	0.86	0.98	0.90	0.96
P, %	0.61	0.64	0.65	0.60	0.56	0.64	0.58	0.59	0.64	0.59	0.64	0.59
Zn, ppm <sup>4</sup>	122	205	194	110	131	193	120	150	183	112	176	226

<sup>1</sup>Multiple samples of each diet were collected, blended and sub sampled, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD).

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

<sup>4</sup>Zinc values represent means from 1 sample at Ward Laboratories Inc., Kearney, NE, and 2 samples at Cumberland Valley Analytical Services, Hagerstown, MD.

**Table 3. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Phase 3						Phase 4					
	ZnSO <sub>4</sub> <sup>2</sup> , ppm			Zn hydroxychloride <sup>3</sup> , ppm			ZnSO <sub>4</sub> <sup>2</sup> , ppm			Zn hydroxychloride <sup>3</sup> , ppm		
	50	100	150	50	100	150	50	100	150	50	100	150
DM, %	87.20	87.10	87.20	87.20	87.10	87.20	87.00	86.6	87.20	86.70	87.10	87.20
CP, %	19.70	21.20	20.30	20.10	19.50	20.40	20.20	20.70	20.20	20.20	20.30	19.90
Crude fiber, %	4.10	4.30	4.00	4.20	4.30	3.70	3.90	4.50	4.20	4.20	4.40	4.20
Ether extract, %	5.10	5.76	5.60	5.51	5.00	4.70	5.64	5.35	5.69	6.09	5.94	5.11
Ash, %	5.80	5.17	5.26	5.28	5.74	5.33	5.13	5.81	5.62	4.98	5.83	5.04
Ca, %	1.00	0.73	0.93	0.90	0.89	1.01	0.74	0.81	0.86	0.66	0.86	0.73
P, %	0.55	0.55	0.54	0.54	0.53	0.52	0.54	0.56	0.57	0.57	0.58	0.57
Zn, ppm <sup>4</sup>	216	178	193	114	158	183	140	178	219	131	128	178

<sup>1</sup>Multiple samples of each diet were collected, blended and sub sampled, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD).

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

<sup>4</sup>Zinc values represents means from 1 sample at Ward Laboratories Inc., Kearney, NE, and 2 samples at Cumberland Valley Analytical Services, Hagerstown, MD.

**Table 4. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Phase 5					
	ZnSO <sub>4</sub> <sup>2</sup> , ppm			Zn hydroxychloride <sup>3</sup> , ppm		
	50	100	150	50	100	150
DM, %	86.10	86.00	85.80	85.70	86.10	86.10
CP, %	19.70	19.50	19.30	18.80	19.10	19.50
Crude fiber, %	3.20	3.40	3.00	3.10	3.10	3.40
Ether extract, %	2.47	3.74	3.90	4.03	3.99	3.97
Ash, %	5.14	6.43	4.79	4.28	5.57	5.07
Ca, %	0.71	0.76	0.70	0.61	0.64	0.73
P, %	0.47	0.46	0.46	0.48	0.49	0.51
Zn, ppm <sup>4</sup>	83	162	225	101	137	204

<sup>1</sup>Multiple samples of each diet were collected, blended and sub sampled, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD).

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

<sup>4</sup>Zinc values represents means from 1 sample at Ward Laboratories Inc., Kearney, NE, and 2 samples at Cumberland Valley Analytical Services, Hagerstown, MD.

**Table 5. Effects of increasing Zn from ZnSO<sub>4</sub> or Zn hydroxychloride on growth performance of pigs<sup>1</sup>**

Item	ZnSO <sub>4</sub> ppm <sup>2</sup>			Zn hydroxychloride, ppm <sup>3</sup>			SEM	Zn source	Probability, <i>P</i> <			
	50	100	150	50	100	150			Zn level		Source × level	
									Linear	Quadratic	Linear	Quadratic
BW, lb												
d 0	70.7	70.7	70.7	70.7	70.6	70.6	0.722	0.899	0.951	0.971	0.951	0.971
d 33	139.3	139.3	140.9	139.9	140.5	138.9	1.040	0.867	0.675	0.823	0.111	0.177
d 66	207.6	208.5	208.9	207.8	209.5	206.2	1.362	0.563	0.904	0.166	0.200	0.242
d 103	278.2	282.7	277.8	280.1	285.4	278.8	2.378	0.326	0.703	0.011	0.848	0.766
d 0 to 33												
ADG, lb	2.07	2.07	2.12	2.09	2.09	2.07	0.024	0.895	0.631	0.742	0.174	0.308
ADFI, lb	4.52	4.50	4.56	4.56	4.55	4.51	0.043	0.720	0.950	0.770	0.318	0.441
F/G	2.19	2.19	2.16	2.19	2.18	2.19	0.017	0.533	0.459	0.869	0.477	0.548
d 33 to 66												
ADG, lb	2.07	2.10	2.06	2.05	2.08	2.04	0.022	0.299	0.659	0.092	0.925	0.886
ADFI, lb	5.50	5.60	5.71	5.49	5.76	5.61	0.078	0.830	0.042	0.130	0.580	0.114
F/G	2.65	2.66	2.76	2.66	2.76	2.74	0.022	0.096	0.001	0.700	0.332	0.007
d 66 to 103												
ADG, lb	2.06	2.17	2.07	2.12	2.24	2.09	0.040	0.112	0.880	0.001	0.649	0.735
ADFI, lb	6.25	6.48	6.22	6.34	6.65	6.63	0.116	0.026	0.251	0.051	0.163	0.689
F/G	3.01	2.93	2.99	2.92	2.92	3.08	0.036	0.840	0.049	0.011	0.007	0.862
d 0 to 103												
ADG, lb	2.07	2.11	2.09	2.09	2.14	2.07	0.020	0.555	0.951	0.007	0.376	0.487
ADFI, lb	5.42	5.52	5.45	5.45	5.64	5.58	0.069	0.163	0.168	0.126	0.660	0.603
F/G	2.62	2.60	2.59	2.59	2.63	2.67	0.018	0.304	0.005	0.265	0.068	0.463

<sup>1</sup>A total of 1,008 pigs (TR4 × (Fast × L02 PIC); initially 70.6 lb) were used with 21 pigs per pen and 8 pens per treatment. The trace mineral premix contributed 1 ppm of Zn to the complete diet.

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

**Table 6. Effects of increasing Zn from ZnSO<sub>4</sub> or Zn hydroxychloride on carcass characteristics of finishing pigs<sup>1</sup>**

Item							Probability, <i>P</i> <					
	ZnSO <sub>4</sub> , ppm <sup>2</sup>			Zn hydroxychloride, ppm <sup>3</sup>			SEM	Zn Source	Level		Source × level	
	50	100	150	50	100	150			Linear	Quadratic	Linear	Quadratic
Yield, %	73.63	74.08	74.53	74.03	74.68	74.36	0.003	0.240	0.027	0.329	0.288	0.327
HCW, lb	204.2	209.5	206.5	208.0	213.6	208.4	1.95	0.041	0.494	0.006	0.618	0.696
Backfat <sup>4</sup> , in.	0.69	0.68	0.70	0.69	0.68	0.68	0.016	0.618	0.802	0.343	0.717	0.445
Loin depth <sup>4</sup> , in.	2.47	2.50	2.49	2.50	2.52	2.47	0.036	0.727	0.947	0.374	0.464	0.845
Lean <sup>4</sup> , %	53.75	54.13	53.80	53.96	54.11	53.93	0.264	0.634	0.975	0.254	0.879	0.678
HCW ADG, lb	1.47	1.52	1.49	1.51	1.56	1.51	0.018	0.036	0.522	0.005	0.669	0.700

<sup>1</sup>A total of 1,008 pigs (TR4 × (Fast × PIC L02); initially 70.6 lb) were used in a 103 d growth study.

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

<sup>4</sup>Hot carcass weight was used as a covariate.

**Table 7. Effects of increasing Zn from ZnSO<sub>4</sub> or Zn hydroxychloride on economic performance<sup>1</sup>**

Item	Zn Source						SEM	Probability, <i>P</i> <				
	ZnSO <sub>4</sub> , ppm <sup>2</sup>			Zn hydroxychloride, ppm <sup>3</sup>				Zn level			Source × level	
	50	100	150	50	100	150		Zn Source	Linear	Quadratic	Linear	Quadratic
Constant day, \$/pig												
Feed cost <sup>4</sup>	45.25	46.16	45.36	45.58	47.38	47.15	0.637	0.038	0.196	0.098	0.259	0.879
Cost/lb gain carcass wt.	0.298	0.295	0.293	0.295	0.297	0.305	0.0024	0.048	0.182	0.350	0.002	0.615
Carcass F/G	3.56	3.52	3.52	3.52	3.55	3.63	0.027	0.132	0.232	0.373	0.005	0.934
Carcass gain value <sup>5</sup>	169.37	172.77	170.20	172.43	177.72	171.83	1.843	0.039	0.948	0.011	0.683	0.416
IOFC	123.91	126.63	125.22	126.56	129.53	124.39	1.245	0.125	0.708	0.007	0.143	0.351
Constant carcass wt, \$/pig <sup>6</sup>												
Adj. carcass F/G <sup>7</sup>	3.63	3.55	3.56	3.55	3.51	3.65	0.035	0.784	0.543	0.022	0.011	0.496
Feed cost	47.40	46.37	46.47	46.26	45.95	48.18	0.437	0.899	0.235	0.020	0.002	0.347
Cost/lb gain carcass wt.	0.301	0.295	0.296	0.296	0.293	0.306	0.0028	0.724	0.304	0.029	0.005	0.375
Carcass gain value	173.21	173.21	173.21	173.21	173.21	173.21	0.000	---	---	---	---	---
IOFC <sup>8</sup>	125.81	126.83	126.74	126.95	127.26	125.03	0.438	0.901	0.239	0.020	0.002	0.346
Facility cost <sup>9</sup>	10.61	10.35	10.55	10.36	9.97	10.42	0.144	0.038	0.972	0.013	0.667	0.448
IOFFC <sup>10</sup>	115.19	116.49	116.19	116.59	117.31	114.62	0.540	0.623	0.334	0.010	0.006	0.328

<sup>1</sup>A total of 1,008 pigs (TR4 × (Fast × L02 PIC); initially 70.6 lb) were used with 21 pigs per pen and 8 pens per treatment. The trace mineral premix contributed 1 ppm of Zn to the complete diet. Carcass price was calculated at \$0.82/lb.

<sup>2</sup>Zinc sulfate (Agrium Advance Technology, Loveland, CO).

<sup>3</sup>Intellibond-Z (Micronutrients, Indianapolis, IN).

<sup>4</sup>Corn, soybean-meal and DDGS were calculated at \$0.05, 0.14 and 0.04/lb, respectively. Test ingredients used were Zn hydroxychloride and ZnSO<sub>4</sub> and calculated at \$2.80 and \$1.10/lb, respectively. Grind, mix and delivery was calculated at \$12.00/ton.

<sup>5</sup>Carcass gain value was calculated using (total carcass gain × carcass price).

<sup>6</sup>Adjusted to constant final carcass weight of 210 lb.

<sup>7</sup>Adjusted using a factor of 0.005 for 1 lb change in carcass weight.

<sup>8</sup>Income over feed cost = carcass gain value – feed cost.

<sup>9</sup>Facility cost at \$0.10/hd/day.

<sup>10</sup>Income over feed and facility cost = IOFC – facility cost.

## Effects of Increasing Levels of Copper from Either $\text{CuSO}_4$ or Combinations of $\text{CuSO}_4$ and a Cu-Amino Acid Complex on Growth Performance, Carcass Characteristics, and Economics of Finishing Pigs<sup>1</sup>

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### Summary

A total of 1,089 pigs (PIC 280 × 1050; initially 82.2 lb) were used in a 105-d experiment to determine the effects of increasing added Cu from either  $\text{CuSO}_4$  alone or a 50/50 blend of  $\text{CuSO}_4$  and Cu-AA (Availa<sup>®</sup>-Cu, Zinpro Corporation, Eden Prairie, MN) on growth performance, carcass characteristics, and economics of finishing pigs. All 6 dietary treatments contained 17 ppm Cu from  $\text{CuSO}_4$  from the trace mineral premix. Additional treatment diets contained added  $\text{CuSO}_4$  to provide 70 and 130 ppm total Cu or a 50/50 blend of added Cu from  $\text{CuSO}_4$  and Cu-AA to provide 70, 100, and 130 ppm total Cu. There were 25 or 26 pigs per pen and 7 replicate pens per treatment.

Overall, added Cu above 17 ppm did not influence ADG; however, pigs fed 70 and 130 ppm added Cu from the 50/50 blend of  $\text{CuSO}_4$  and Cu-AA had decreased ( $P = 0.045$ ) ADFI and improved feed efficiency ( $P = 0.048$ ) compared with those fed 70 and 130 ppm of added Cu from  $\text{CuSO}_4$  only. Similar to the F/G response, pigs fed diets that contained  $\text{CuSO}_4$  alone had poorer ( $P = 0.030$ ) carcass F/G than those fed added Cu from the 50/50 blend of  $\text{CuSO}_4$  and Cu-AA. Neither Cu source nor level influenced economics.

In conclusion, these data suggest pigs fed diets that contained added Cu from  $\text{CuSO}_4$  alone consume more feed but have poorer feed efficiency which translates into poorer carcass F/G compared to those fed a 50/50 blend of  $\text{CuSO}_4$  and Cu-AA. Copper level did not impact growth performance. Based on our study, it appears that the 50/50

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blend of  $\text{CuSO}_4$ /Cu-AA optimized feed efficiency and carcass feed efficiency of pigs marketed on a constant time basis.

Key words: carcass characteristics, copper, finishing pig, growth, level, source

## Introduction

Feeding high concentrations of Cu from  $\text{CuSO}_4$  has been associated with improved growth performance of growing pigs. However, the responses observed in different trials are variable and may depend on feeding period or concentration. Coble et al. (2015)<sup>4</sup> reported ADG tended to increase when pigs were fed added Cu from tri-basic copper chloride during the early finishing period. However, Feldpausch et al. (2015)<sup>5</sup> reported no growth promoting benefit of 150 ppm added Cu from  $\text{CuSO}_4$  during either the early or late finishing periods. Further investigation is warranted to better understand how high levels of Cu will impact growing and finishing pig performance. Furthermore, it is not well understood if the specific source of Cu will lead to differences in pig performance. Therefore, the objective of this study was to determine the effects of increasing Cu provided from either  $\text{CuSO}_4$  alone or a 50/50 blend of  $\text{CuSO}_4$  and Cu-AA on growth performance, carcass characteristics, and economics of finishing pigs housed in a commercial environment.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted in a commercial research facility in southwestern Minnesota. The facility was double-curtain-sided with completely slatted concrete flooring. The barn contained 42 pens with 25 or 26 pigs (mixed gender) in each, equipped with a 4-hole conventional dry self-feeder (Thorp Equipment, Thorp, WI) and 1 cup-waterer, providing ad libitum access to feed and water. A computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) delivered and recorded daily feed additions of each diet to the respective pen.

A total of 1,089 pigs (PIC 280 × 1050; initially 82.2 lb) were used in a 105-d experiment to determine the effects of increasing Cu provided from either  $\text{CuSO}_4$  alone or a 50/50 blend of  $\text{CuSO}_4$  and Cu-AA (Availa-Cu, Zinpro Corporation, Eden Prairie, MN) on growth performance, carcass characteristics, and economics of finishing pigs. On d 0, pens of pigs were weighed, blocked by average pig BW, and randomly allotted to 1 of 6 dietary treatments. There were 7 replicate pens per treatment. The 6 dietary treatments consisted of a control diet which contained 17 ppm Cu from  $\text{CuSO}_4$  from the trace mineral premix, or the control diet with either added  $\text{CuSO}_4$  to provide 70

<sup>4</sup> Coble, K. F.; Burnett, D. D.; Goodband, R. D.; Gonzales, J. M.; Usry, J.; Tokach, M. D.; Pluske, J. R.; DeRouchey, J. M.; Woodworth, J. C.; Dritz, S. S.; Flohr, J. R.; and Vaughn, M. A. (2015) "Effect of Diet Type and Added Copper on Growth Performance, Carcass Characteristics, Energy Digestibility, Gut Morphology, and Mucosal mRNA Expression of Finishing Pigs," Kansas Agricultural Experiment Station Research Reports: Vol. 1: Iss. 7.

<sup>5</sup> Feldpausch, J. A.; Amachawadi, R. G.; Scott, H. M.; Tokach, M. D.; Dritz, S. S.; Woodworth, J. C.; Nagaraja, T. G.; Goodband, R. D.; and DeRouchey, J. M. (2015) "Effects of Added Copper and Zinc on Growth Performance and Carcass Characteristics of Finishing Pigs Fed Diets with or without Ractopamine HCl," Kansas Agricultural Experiment Station Research Reports: Vol. 1: Iss. 7.

and 130 ppm total Cu, or a 50/50 blend of Cu from  $\text{CuSO}_4$  and Cu-AA to provide 70, 100, and 130 ppm total Cu.

Experimental diets were fed in 5 phases (approximately 80 to 100, 100 to 135, 135 to 170, 170 to 230, and 230 to 280 lb). For diets that contained added Cu above that provided from the trace mineral premix, Cu was added at the expense of corn. Nutrient values for the ingredients were based on the NRC (2012)<sup>6</sup>. Diets were fed in meal form and were manufactured at the New Horizon Feed Mill (Pipestone, MN).

Complete diet samples were collected from a minimum of 6 feeders per phase and combined to make 1 composite sample per treatment within phase. Each sample was then split, ground and then sent to Minnesota Valley Testing Laboratories (New Ulm, MN) for analysis of DM, CP, ash, Ca, P, and Cu concentrations (Table 2, 3 and 4).

Pigs were weighed and feed disappearance was measured approximately every 2 weeks to calculate ADG, ADFI, and F/G. On d 79 of the trial, pens were weighed and the 3 heaviest pigs from each pen were removed and transported 59 miles to JBS USA (Worthington, MN) for harvest. These pigs were used in calculation of pen growth performance, but not carcass characteristics.

On d 105, final pen weights were recorded and feed disappearance was measured. The remaining pigs in the barn were individually tattooed with a pen identification number to allow individual carcass measurements to be recorded, and transported to the same aforementioned harvest facility for carcass data collection. Carcass yield was calculated using HCW at the plant divided by average individual live weight at the farm. Standard carcass measurements of backfat (BF), loin depth (LD), and percentage lean (Lean, %) were measured, with pen as experimental unit and carcass as the observational unit. Fat depth and loin depth were measured with an optical probe [Fat-O-Meter (SFK, Herlev, Denmark)] inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 2.76 in. from the dorsal midline.

Economic comparisons were made based on both a constant ending weight and a constant day basis. Total feed cost per pig, cost per pound of gain, carcass ADG, F/G, carcass gain value, and income over feed cost (IOFC) were calculated. An assumed carcass yield of 75% was used to calculate initial HCW at the beginning of the experiment. Hot carcass weight ADG was calculated by subtracting initial HCW from the final HCW obtained at the plant, then divided by 105 d on test. Hot carcass weight F/G was calculated by dividing the pen total feed intake by pen total carcass weight gain. Feed cost was calculated by multiplying total feed intake per pig by a weighted mean diet cost on a per pen basis. Prices used for corn, soybean-meal, and DDGS at the time of the experiment were \$0.06, 0.14, and 0.05/lb, respectively. Prices used for the Cu-AA and  $\text{CuSO}_4$  were \$2.14 and \$1.00/lb, respectively. Carcass price at time of slaughter was calculated at \$0.74 per pound. Cost per pound of gain was calculated by dividing the total feed cost per pig by the total carcass pounds gained overall. The value of the carcass weight gained during the experiment (gain value) was calculated by multiplying the carcass value by the pen final carcass weight. Income over feed cost was calculated by subtracting total feed cost from gain value. The income over feed and facilities cost

<sup>6</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, D.C.

(IOFFC) was calculated for the constant market weight evaluation because pigs with faster growth rates will reach a 210 lb carcass sooner, therefore decreasing housing costs. Facility cost was calculated by multiplying the number of overall days the pigs need to reach a 210 lb carcass based on their respective growth rate by \$0.11 per head per day facility cost.

Data were analyzed as a randomized complete block design using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Hot carcass weight was used as a covariate for carcass characteristics including percentage lean, loin depth, and backfat. Effects of Cu source and linear and quadratic effects of Cu level were analyzed with significance defined as  $P \leq 0.05$  and marginally significant as  $P > 0.05$  and  $\leq 0.10$ .

## Results and Discussion

The chemical analyses of the complete diets were similar to the intended formulation (Tables 1, 2, 3, and 4). Total Ca and P levels were similar among diets across each dietary phase. The total analyzed Cu concentrations for diets formulated to 17, 70, and 130 ppm total Cu from  $\text{CuSO}_4$  ranged from 27 to 58, 62 to 94, and 46 to 133 ppm, respectively. Total analyzed Cu concentrations for diets formulated to 70, 100, and 130 ppm total Cu from  $\text{CuSO}_4/\text{Cu-AA}$  ranged from 69 to 130, 80 to 119, and 98 to 142 ppm, respectively.

Of the 30 experimental diets, 6 diets were outside the analytical variation limits for Cu (25%, AAFCO, 2014)<sup>7</sup>. In Phase 1, the diet formulated to contain 70 ppm Cu from  $\text{CuSO}_4$  was slightly lower and the diet formulated to contain 130 ppm Cu from the 50/50 blend was lower in analyzed Cu concentration than expected. In Phase 2, the control diet was slightly higher in analyzed Cu than expected. In Phase 3, the control diet and the diet formulated to contain 70 ppm Cu from the 50/50 blend were higher in analyzed Cu than expected and the diet formulated to contain 130 ppm Cu from  $\text{CuSO}_4$  alone was much lower in analyzed Cu than expected.

All other total Cu values for each diet were within the acceptable analytical limits described by the AAFCO (2014) given that 17 ppm of Cu from  $\text{CuSO}_4$  was provided by the trace mineral premix and accounting for the Cu provided by ingredients used in formulation. Corn, soybean meal, and corn DDGS can contain on average 15, 50, and 52 ppm Cu, respectively (NRC, 2012). Based on these Cu concentrations, corn, soybean meal and corn DDGS may have contributed up to 14 ppm Cu to the complete diet in our study. Thus, some of the variation observed in the Cu analysis may partially be explained by the Cu concentrations provided by major ingredients used in formulation.

From d 0 to 43, neither Cu source nor level influenced growth performance (Table 5).

From d 43 to 105, ADFI was lower ( $P = 0.037$ ) for pigs fed the 50/50 blend of added Cu from  $\text{CuSO}_4$  and Cu-AA compared to those fed added Cu from  $\text{CuSO}_4$  alone. Feed efficiency tended to be improved (linear,  $P = 0.057$ ) as level of Cu increased.

<sup>7</sup> Association of American Feed Control Officials (AAFCO). 2014. Official Publication. Assoc. Am. Feed Cont. Off., Champaign, IL.

Overall, d 0 to 105, neither Cu level nor source influenced ADG. Pigs fed 70 and 130 ppm added Cu from the 50/50 blend of CuSO<sub>4</sub> and Cu-AA had lower ( $P = 0.045$ ) ADFI and improved feed efficiency ( $P = 0.048$ ) compared with those fed the same amount of added Cu from only CuSO<sub>4</sub>. Due to the decreased ADFI and improved F/G of pigs fed the 50/50 blend of added Cu from CuSO<sub>4</sub> and Cu-AA, carcass F/G also improved ( $P = 0.030$ ; Table 6) compared with those fed added Cu from CuSO<sub>4</sub> alone.

Regarding economics, neither Cu source nor level influenced economics when reported on a constant time or constant weight basis (Table 7).

Although there are limited data available describing the effects of Cu blends, a variety of experiments have demonstrated conflicting results on the growth-promoting benefits of added Cu above that provided by the trace mineral premix. Hastad et al. (2001)<sup>8</sup> reported there were no growth benefits above 135 lb of BW for pigs fed diets that contained 50, 100, or 200 ppm added Cu from CuSO<sub>4</sub>. However, much of our data agree with similar experiments that have compared the effects of inorganic and organic sources of Cu. Previously, Coble et al. (2014)<sup>9</sup> used CuSO<sub>4</sub> and an organic Cu chelate (Mintrex Cu) and reported no differences in ADG. In their study, pigs fed diets that contained either 50 or 125 ppm of added Cu from CuSO<sub>4</sub> throughout the entire experiment had greater ADFI but poorer feed efficiency than the control. This resulted in poorer F/G for pigs fed CuSO<sub>4</sub> throughout the experiment. These results were similar to our study in that although pigs fed added CuSO<sub>4</sub> consumed more feed, they had poorer F/G due to the lack of a gain response. In addition, Coble et al. (2014) also reported pigs fed diets that contained CuSO<sub>4</sub> had poorer carcass F/G which supports the current study's findings. Although the study herein and Coble et al. (2014) demonstrated intake was higher for pigs fed Cu from CuSO<sub>4</sub>, Feldpausch et al. (2015)<sup>10</sup> added 125 ppm of Cu from CuSO<sub>4</sub> and did not observe any differences in growth or carcass characteristics.

In summary, our study suggests differences exist between feeding added Cu as either a blend or single source on growth performance, carcass characteristics or economics. These data suggest pigs fed diets that contain added Cu from CuSO<sub>4</sub> had greater ADFI but are less efficient. Furthermore, carcass F/G worsened when diets contained CuSO<sub>4</sub> compared to those fed a 50/50 blend of CuSO<sub>4</sub> and Cu-AA, which is likely explained by the poorer F/G of pigs fed CuSO<sub>4</sub> alone. Our data suggest a 50/50 blend of CuSO<sub>4</sub> and Cu-AA has the potential to improve F/G as a result of reduced feed intake but no difference in overall gain or ending BW. Based on our study, it appears a 50/50 blend of CuSO<sub>4</sub> and Cu-AA optimizes feed efficiency and carcass feed efficiency for pigs marketed on a constant time basis.

<sup>8</sup> Hastad et al., Swine Day 2001. Report of Progress 880, pp. 111–117. Kansas Agricultural Experiment Station, Manhattan, KS.

<sup>9</sup> Coble et al., Swine Day 2014. Report of Progress 1110, pp. 155-163. Kansas Agricultural Experiment Station, Manhattan, KS.

<sup>10</sup> Feldpausch, J. A.; Amachawadi, R. G.; Scott, H. M.; Tokach, M. D.; Dritz, S. S.; Woodworth, J. C.; Nagaraja, T. G.; Goodband, R. D. and DeRouchey, J. M. (2015) "Effects of Added Copper and Zinc on Growth Performance and Carcass Characteristics of Finishing Pigs Fed Diets with or without Ractopamine HCl," Kansas Agricultural Experiment Station Research Reports: Vol. 1: Iss. 7.

**Table 1. Diet composition (as-fed basis)**

Item	Phase <sup>1,2</sup>				
	1	2	3	4	5
Ingredient, %					
Corn	56.04	61.33	65.87	69.32	79.48
Soybean meal (46.0 % CP)	21.61	16.52	11.97	8.52	8.39
DDGS	20.00	20.00	20.00	20.00	10.00
Calcium carbonate	1.25	1.20	1.18	1.15	1.13
Monocalcium P (21.5% P)	0.15	---	---	---	0.09
Salt	0.35	0.35	0.35	0.35	0.35
L-Lys-HCl	0.36	0.37	0.39	0.39	0.32
DL-Met	0.01	---	---	---	---
L-Thr	0.05	0.04	0.05	0.06	0.07
L-Trp	---	0.01	0.02	0.02	0.02
Optiphos 2000 <sup>3</sup>	0.01	0.01	0.01	0.01	0.01
Trace mineral premix <sup>4</sup>	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>4</sup>	0.08	0.08	0.08	0.08	0.05
Cu Source <sup>5</sup>	---	---	---	---	---
Total	100.00	100.00	100.00	100.00	100.00

*continued*

**Table 1. Diet composition (as-fed basis)**

Item	Phase <sup>1,2</sup>				
	1	2	3	4	5
Calculated analysis					
Standardized ileal digestible (SID) AA, %					
Lys	1.02	0.91	0.82	0.74	0.65
Ile:Lys	63	62	60	59	59
Leu:Lys	152	159	164	171	166
Met:Lys	29	29	30	31	30
Met + Cys:Lys	55	56	57	59	59
Thr:Lys	61	61	61	63	65
Trp:Lys	18.4	18.5	18.5	18.5	18.5
Val:Lys	70	70	70	70	70
Total Lys, %	1.18	1.06	0.96	0.87	0.76
ME, kcal/lb	1,502	1,508	1,510	1,512	1,511
NE, kcal/lb	1,103	1,119	1,131	1,141	1,155
SID Lys:ME, g/Mcal	5.28	4.62	4.10	3.66	2.84
CP, %	20.02	18.08	16.36	15.05	12.94
Ca, %	0.61	0.55	0.52	0.50	0.50
P, %	0.45	0.40	0.38	0.36	0.34
Available P, %	0.29	0.26	0.25	0.25	0.22

<sup>1</sup>Phases 1, 2, 3, 4, and 5 were fed from d 0 to 9, 9 to 28, 28 to 43, 43 to 72, and 72 to 105, respectively.

<sup>2</sup>The basal diet contained 17 ppm Cu from CuSO<sub>4</sub>.

<sup>3</sup>Optiphos 2000 (Huvepharma, Peach Tree, GA) provided 568 phytase units (FTU)/lb with a release of 0.10% available P.

<sup>4</sup>The vitamin premix supplied vitamin A 3,200,000 I.U., vitamin D3 500,000 I.U., vitamin E 16,000 I.U., vitamin (B12) 12 mg, riboflavin (B2) 2,800 mg, niacin 18,000 mg, d-pantothenic acid 10,000 mg, menadione 1600 mg. The trace mineral premix supplied Zn 110 ppm, Fe 110 ppm, Mn 33 ppm, Cu 17 ppm, I 0.33 ppm, and Se 0.30 ppm. Vitamin concentrations are expressed on a per lb of product basis whereas minerals are expressed on a ppm basis.

<sup>5</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products Inc., Quincy, IL) or Availa<sup>®</sup> Cu (Cu-AA; Zinpro Corporation, Eden Prairie, MN). All experimental diets contained the basal diet and added Cu from either CuSO<sub>4</sub> only or a 50/50 blend of CuSO<sub>4</sub> and Cu-AA. For diets containing CuSO<sub>4</sub> only, either 0, 53 or 113 ppm of additional Cu from CuSO<sub>4</sub> was added at the expense of corn. For diets containing a 50/50 blend of CuSO<sub>4</sub> and Cu-AA, each diet was formed by adding additional Cu at either 18 and 35, 33 and 50, or 48 and 65 ppm from CuSO<sub>4</sub> and Cu-AA, respectively, at the expense of corn.

**Table 2. Chemical analysis of diets (as-fed basis)**

Item	Phase 1						Phase 2					
	Added Cu, ppm						Added Cu, ppm					
	Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm <sup>5</sup>			Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm <sup>5</sup>		
	0	70	130	70	100	130	0	70	130	70	100	130
DM, %	86.35	86.29	85.27	86.31	86.32	86.29	86.34	85.88	86.00	86.23	85.94	85.80
CP, %	20.70	20.50	20.50	20.10	20.20	20.30	19.40	19.20	17.60	19.90	18.20	18.30
Ash, %	4.38	4.50	4.44	5.06	3.78	4.22	4.20	3.88	3.93	3.78	3.96	3.77
Ca, %	0.61	0.58	0.42	0.85	0.62	0.57	0.59	0.46	0.56	0.45	0.62	0.51
P, %	0.51	0.50	0.55	0.50	0.51	0.51	0.46	0.48	0.45	0.46	0.45	0.45
Cu, ppm <sup>6</sup>	27	62	131	100	99	98	40	78	117	69	88	120

<sup>1</sup>Multiple samples of each diet were collected, blended and sub-sampled before being analyzed at Minnesota Valley Testing Laboratory (New Ulm, MN).

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products, Quincy, IL).

<sup>4</sup>Availa<sup>®</sup> Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Copper concentration was achieved by a 50/50 inclusion of each copper source.

<sup>6</sup>Copper values represent means from 2 individual samples analyzed 1 or 2 times at Minnesota Valley Testing Laboratories (New Ulm, MN).

**Table 3. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Phase 3						Phase 4					
	Added Cu, ppm						Added Cu, ppm					
	Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm <sup>5</sup>			Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm <sup>5</sup>		
0	70	130	70	100	130	0	70	130	70	100	130	
DM, %	85.74	86.06	86.21	86.21	85.89	86.12	85.97	86.13	86.03	86.18	85.90	85.81
CP, %	16.50	15.90	15.20	16.70	16.10	16.20	13.30	13.50	13.70	14.50	15.30	15.80
Ash, %	3.52	3.61	3.61	3.48	3.47	3.57	3.34	3.38	3.35	3.46	3.44	3.62
Ca, %	0.45	0.61	0.63	0.49	0.47	0.55	0.55	0.60	0.58	0.54	0.48	0.55
P, %	0.41	0.39	0.37	0.40	0.41	0.40	0.36	0.36	0.36	0.36	0.40	0.41
Cu, ppm <sup>6</sup>	58	73	46	130	80	109	31	80	133	89	119	137

<sup>1</sup>Multiple samples of each diet were collected, blended and sub-sampled before being analyzed at Minnesota Valley Testing Laboratory (New Ulm, MN).

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products, Quincy, IL).

<sup>4</sup>Availa<sup>®</sup> Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Copper concentration was achieved by a 50/50 inclusion of each copper source.

<sup>6</sup>Copper values represent means from 2 individual samples analyzed 1 or 2 times at Minnesota Valley Testing Laboratories (New Ulm, MN).

**Table 4. Chemical analysis of diets (as-fed basis)<sup>1</sup>**

Item	Phase 5					
	Added Cu, ppm					
	Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm <sup>5</sup>		
	0	70	130	70	100	130
DM, %	85.93	85.86	85.98	86.09	86.17	85.72
CP, %	13.70	13.50	14.00	13.50	13.30	13.80
Ash, %	3.40	3.43	3.29	3.41	3.11	3.15
Ca, %	0.63	0.62	0.55	0.66	0.51	0.51
P, %	0.35	0.38	0.37	0.37	0.36	0.39
Cu, ppm <sup>6</sup>	31	94	110	89	115	142

<sup>1</sup>Multiple samples of each diet were collected, blended and sub-sampled before being analyzed at Minnesota Valley Testing Laboratory (New Ulm, MN).

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products, Quincy, IL).

<sup>4</sup>Availa<sup>®</sup> Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Copper concentration was achieved by a 50/50 inclusion of each copper source.

<sup>6</sup>Copper values represent means from 2 individual samples analyzed 1 or 2 times at Minnesota Valley Testing Laboratories (New Ulm, MN).

**Table 5. Effects of increasing Cu from either CuSO<sub>4</sub> or combinations of CuSO<sub>4</sub> and Cu-AA on finishing pig growth performance<sup>1</sup>**

Item	Control <sup>2</sup> 17	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm			SEM	Probability, <i>P</i> <		
		70	130	70	100	130		Cu Source <sup>5</sup>	Cu level Linear	Quadratic
BW, lb										
d 0	82.0	82.0	82.3	82.0	82.6	82.0	2.48	0.848	0.748	0.867
d 43	169.4	170.1	171.7	171.0	172.2	170.4	3.75	0.880	0.292	0.559
d 105	281.6	285.3	285.9	284.3	287.7	282.9	4.01	0.467	0.247	0.235
d 0 to 43										
ADG, lb	2.03	2.05	2.07	2.07	2.08	2.05	0.035	0.936	0.264	0.408
ADFI, lb	4.71	4.77	4.83	4.78	4.87	4.71	0.086	0.321	0.186	0.142
F/G	2.32	2.33	2.33	2.31	2.34	2.29	0.022	0.169	0.945	0.505
d 43 to 105										
ADG, lb	1.83	1.87	1.88	1.86	1.87	1.83	0.028	0.400	0.455	0.334
ADFI, lb	5.82	5.90	5.89	5.82	5.83	5.65	0.075	0.037	0.603	0.349
F/G	3.18	3.16	3.14	3.12	3.12	3.08	0.030	0.110	0.057	0.807
d 0 to 105										
ADG, lb	1.92	1.95	1.96	1.95	1.96	1.93	0.022	0.573	0.249	0.264
ADFI, lb	5.35	5.42	5.44	5.38	5.42	5.24	0.064	0.045	0.916	0.208
F/G	2.79	2.79	2.78	2.76	2.76	2.72	0.022	0.048	0.124	0.925

<sup>1</sup>A total of 1,089 pigs (PIC 280 × 1050; initially 82.2 lb) were used with 25 or 26 pigs per pen and 7 pens per treatment in a 105-d growth study.

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products, Quincy, IL).

<sup>4</sup>Availa<sup>®</sup> Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Main effect of Cu source (70 and 130 ppm, within source).

**Table 6. Effects of increasing Cu from either CuSO<sub>4</sub> or combinations of CuSO<sub>4</sub> and Cu-AA on finishing pig carcass characteristics<sup>1</sup>**

Item	Control <sup>2</sup>	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm			SEM	Probability, <i>P</i> <		
		70	130	70	100	130		Cu level		
								Cu Source <sup>5</sup>	Linear	Quadratic
Yield, %	72.36	72.57	71.91	72.66	72.61	72.44	0.333	0.329	0.796	0.179
HCW, lb	205.1	206.9	207.0	206.6	208.8	204.8	2.98	0.547	0.493	0.247
Backfat <sup>6</sup> , in.	0.68	0.69	0.68	0.69	0.68	0.67	0.014	0.836	0.687	0.770
Loin depth <sup>6</sup> , in.	2.51	2.50	2.49	2.51	2.49	2.57	0.042	0.201	0.790	0.617
Lean <sup>6</sup> , %	55.91	55.84	55.82	55.81	55.98	56.22	0.264	0.363	0.605	0.581
HCW ADG, lb	1.37	1.38	1.38	1.38	1.40	1.37	0.017	0.552	0.519	0.229
Carcass F/G <sup>7</sup>	3.86	3.84	3.86	3.79	3.81	3.76	0.037	0.030	0.221	0.543
Adj. Carcass F/G <sup>8</sup>	3.98	3.92	3.98	3.87	3.87	3.86	0.059	0.143	0.285	0.233

<sup>1</sup>A total of 1,089 pigs (PIC 280 × 1050; initially 82.2 lb) were used with 25 or 26 pigs per pen and 7 pens per treatment in a 105-d growth study.

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>; Prince Agri. Products., Quincy, IL).

<sup>4</sup>Availa<sup>®</sup> Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Main effect of Cu source (70 and 130 ppm, within source).

<sup>6</sup>Hot carcass weight was used as a covariate.

<sup>7</sup>Constant time basis

<sup>8</sup>Adjusted to constant final carcass weight of 210 lb. Adjusted using a factor of 0.005 for 1 lb change in carcass weight.

**Table 7. Effects of increasing Cu from either CuSO<sub>4</sub> or combinations of CuSO<sub>4</sub> and Cu-AA on finishing pig economics<sup>1</sup>**

Item	Control <sup>2</sup> 17	CuSO <sub>4</sub> <sup>3</sup> , ppm		CuSO <sub>4</sub> /Cu-AA <sup>4</sup> , ppm			SEM	Probability, <i>P</i> <		
		70	130	70	100	130		Cu level		
						Cu Source <sup>5</sup>		Linear	Quadratic	
<b>Constant day, \$/pig</b>										
Feed cost <sup>6</sup>	44.91	45.80	45.83	45.42	46.41	44.92	0.583	0.238	0.239	0.110
Cost/lb gain carcass wt.	0.316	0.315	0.319	0.313	0.316	0.313	0.0036	0.274	0.896	0.604
Carcass gain value <sup>7</sup>	151.78	154.13	153.04	153.81	155.53	152.57	2.337	0.814	0.382	0.122
IOFC <sup>8</sup>	106.87	108.33	107.22	108.39	109.12	107.66	1.928	0.849	0.529	0.186
<b>Constant carcass wt, \$/pig<sup>9</sup></b>										
Feed cost	47.65	47.08	47.79	46.89	46.88	47.12	1.009	0.525	0.694	0.295
Cost/lb gain carcass wt.	0.321	0.317	0.323	0.316	0.317	0.317	0.0048	0.471	0.824	0.334
Carcass gain value	156.35	156.35	156.35	156.35	156.35	156.35	---	---	---	---
IOFC	108.69	109.26	108.56	109.46	109.47	109.23	1.009	0.525	0.694	0.295
Facility cost <sup>10</sup>	12.06	11.79	11.91	11.84	11.64	11.96	0.250	0.775	0.342	0.116
IOFFC <sup>11</sup>	96.63	97.48	96.65	97.62	97.82	97.27	1.245	0.648	0.601	0.235

<sup>1</sup>A total of 1,089 pigs (PIC 280 × 1050; initially 82.2 lb) were used with 25 or 26 pigs per pen and 7 pens per treatment in a 105-d growth study. All economics were calculated based on a carcass price of 0.74 \$/lb.

<sup>2</sup>The trace mineral premix was formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> to the complete basal diet.

<sup>3</sup>Copper sulfate (CuSO<sub>4</sub>) (Prince Agri. Products., Quincy, IL).

<sup>4</sup>Availa® Cu (Zinpro Corporation, Eden Prairie, MN).

<sup>5</sup>Main effect of Cu source (70 and 130 ppm, within source).

<sup>6</sup>Corn, soybean-meal and DDGS were calculated at 0.06, 0.17 and 0.05 \$/lb, respectively. Test ingredients used were Cu-AA (Availa® Cu) and CuSO<sub>4</sub> and calculated at 2.14 and 1.00 \$/lb, respectively.

<sup>7</sup>Carcass gain value calculated using (total carcass gain × carcass price).

<sup>8</sup>Income over feed cost = carcass gain value – feed cost.

<sup>9</sup>Adjusted to constant final carcass weight of 210 lb.

<sup>10</sup>Facility cost at 0.11 \$/hd/day.

<sup>11</sup>Income over feed and facility cost = IOFC – facility cost.

## Effect of Sample Preparation and Extended Mix Times with Different Salt Particle Sizes on the Uniformity of Mix of a Corn-Soybean Meal Swine Diet

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### Summary

The uniformity of a feed mixture is determined from the coefficient of variation (CV) of 10 samples in a single batch of feed. The feed industry standard is a CV of less than 10% using a single source tracer, such as salt, trace minerals, or iron filings. The objectives of these experiments were to determine the effects of 1) extended mix time, 2) particle size of the marker, and 3) sample preparation on the CV in a corn-soybean meal swine diet. In Experiment 1, treatments were arranged in a  $3 \times 7$  factorial with main effects of 3 salt particle sizes (fine-350  $\mu\text{m}$ , medium-464  $\mu\text{m}$ , and coarse-728  $\mu\text{m}$ ) and 7 mix times (2, 3, 5, 15, 30, 45, and 60 min). In Experiment 2, treatments were arranged in  $2 \times 3 \times 3$  factorial with 2 sample preparations (unground vs. ground), 3 salt particle sizes (fine-350  $\mu\text{m}$ , medium-464  $\mu\text{m}$ , and coarse-728  $\mu\text{m}$ ) and 3 mix times (3, 30, and 60 min). There were 3 replicates per treatment and 10 samples per replicate. Salt concentrations were determined using a Quantab® Chloride Titrator. The result of Experiment 1 indicated no interaction between mix time and salt particle size. The extended mix time did not result in segregation ( $P = 0.307$ ). Particle size of the salt significantly affected the uniformity of mix ( $P < 0.0001$ ; 21.2, 8.6, and 7.9% CV for the coarse, medium, and fine salt, respectively). The results of Experiment 2 indicated no interaction of sample preparation, salt particle size, and mix time. However, there was interaction between sample preparation and salt particle size ( $P = 0.0002$ ). The difference in the CV% between unground and ground samples was significantly greater for the mixture with coarse salt (8.89%) than the mixture with fine (1.35%) and medium salt (2.59%). The ground treatment had a significantly lower CV than the unground treatment ( $P < 0.0001$ ; 8.7 and 13.0 for ground and unground samples, respectively). The fine and medium salt treatments had significantly lower CV as compared to the coarse salt treatment. ( $P < 0.0001$ ; 7.4, 7.7, and 17.4 for fine, medium and coarse, respectively). These results indicated that feed did not segregate after mixing for up to 1 h. The greater number of particles per gram of the marker (in this case salt) increased the precision of the analysis, likely due to an increased probability that the marker was present in proportionate quantities in the sample tested. However, when coarse salt is used in the manufacturing process, the samples should be ground prior to analysis.

Key words: mix time, particle size, sample preparation, uniformity of mix

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## Introduction

The composition of a diet directly impacts growth rate and feed conversion of animals. Nutritionists formulate diets based on the assumption that animals will receive all the nutrients needed for maintenance and growth each time they go to the feeder. Researchers have demonstrated that poorly mixed feed can negatively affect the feed conversion of nursery pigs (Traylor et al., 1994).<sup>3</sup> In addition to meeting the nutrient requirements of the animal, variation within a mixture of feed can lead to toxicity or deficiency of minerals and vitamins. Several factors can affect the uniformity of mix, such as mixer design, particle size of the ingredients, and mixing time. The goal of the feed mixing process is to create a uniform mixture of feed in the minimum amount of time, in order to maximize process efficiency. However, there is also a concern that extended mixing may lead to ingredient segregation due to inherent sifting by weight or static charge generated during the mixing process. There are little data available to confirm if overmixing, indeed, does lead to segregation.

The selection of an appropriate tracer is also important when determining the uniformity of the mix. Groesbeck et al. (2004)<sup>4</sup> conducted two experiments to determine the effect of various salt particle sizes and different sample preparation on mixing uniformity. The researchers observed an interaction CV decreased when the particle size of the salt decreased from 3,000 to 440 microns, mix time was increased, and samples were ground. However, this has not been considered when evaluating mixing time. The objectives of the current study were to determine the effect of extended mixing time, salt particle size, and sample preparation on the uniformity of mix.

## Procedures

### *Materials and Methods*

#### Experiment 1

Treatments were arranged in a  $3 \times 7$  factorial with main effects of salt particle size (fine-350  $\mu\text{m}$ , medium-464  $\mu\text{m}$ , and coarse-728  $\mu\text{m}$ ) and mix time (2, 3, 5, 15, 30, 45, and 60 min) to determine their effect on uniformity of mix. A swine growing diet was mixed in a 2-ft<sup>3</sup> double ribbon mixer (Hayes and Stolz model HP2SSS-0106, Fort Worth, TX). A total of 10 samples were obtained from 10 different locations in the mixer. There were 3 replicates per treatment. The salt concentration of the samples was analyzed using Chloride Quantab<sup>®</sup> Test Strips (Hach #2751340, Loveland, CO) using the method described by McCoy (2005).<sup>5</sup>

#### Experiment 2

Treatments were arranged in  $2 \times 3 \times 3$  factorial of sample preparation (unground and ground), salt particle size (fine-350  $\mu\text{m}$ , medium-464  $\mu\text{m}$ , and coarse-728  $\mu\text{m}$ ), and mix time (3, 30, and 60 min) to determine their effect on uniformity of mix. A swine growing diet was mixed in the same 2-ft<sup>3</sup> double ribbon mixer used in Experiment 1. The diet was mixed for 3, 30 and 60 min. A total of 10 samples were obtained from 10 different locations in the mixer. The samples were ground with a coffee bean grinder for 30 sec. There were 3 replicates per treatment.

<sup>3</sup> Traylor, S.L., Hancock, J.D., Behnke, K.C., Stark, C.R. and Hines, R.H. 1994. Mix time affects diet uniformity and growth performance of nursery and finishing pigs. In KSU Swine Day Report, 171-175. Kansas State University, Manhattan, KS.

<sup>4</sup> Groesbeck, C., R. D. Goodband, M. D. Tokach, J. L. Nelssen, J. M. DeRouche, and S. S. Dritz. Effects of salt particle size and sample preparation on results of mixer-efficiency testing. 2004 Kansas State University Swine Day Report, 177-181.

<sup>5</sup> McCoy, R. A. 2005. Chapter Appendix D: Mixer Testing. In Feed manufacturing technology V, 620-622. ed. E. K. Schofield and American Feed Industry Association, Arlington, VA. American Feed Industry Association.

### *Data Collection*

The salt concentration of the samples was determined with the Quantab® chloride titrator method. A 10 g sample was weighed into a cup, and 90 g of hot distilled water (60°C) was added to the cup. The mixture was stirred for 30 sec, allowed to rest for 60 sec, and stirred for another 30 sec. A folded filter paper was placed into the cup and the Quantab® strip was inserted into the liquid at the bottom of the filter paper. The result of 10 samples per batch were used to compute a coefficient of variation to determine mixing uniformity. The coefficient of variation for each batch was calculated by dividing the standard deviation by the average value multiplied with 100. The particle size of the 3 types of salt (fine, medium and coarse) was determined with a Ro-Tap model RX-29 (W.S. Tyler Industrial Group, Mentor, OH) using the method of determining and expressing fineness of feed materials by sieving (ANSI/ASAE S319.4) without a flow agent for 15 min.

### *Statistical Analysis*

Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means adjustment for Bonferroni's multiple comparisons. Results were considered significant if  $P \leq 0.05$ .

## **Results and Discussion**

### *Experiment 1*

There was no interaction between mixing time and particle size of the salt. Mix time did not affect CV (Table 1), and suggested that salt particles did not segregate during the extended mixing process. However, mixing with coarse salt increased CV compared to the fine and medium salt treatments ( $P < 0.0001$ : 7.9, 8.6, and 21.2 for fine, medium, and coarse, respectively). Coefficient of variation decreased as the number of marker particles (salt) per gram increased. Notably, there was a decline in CV when the mixture included coarse salt after 2 minutes of mix time. This reduction may be due to a reduction in salt particle size due to friction between particles.

### *Experiment 2*

There was no interaction observed between sample preparation, salt particle size, and mix time. However, there was a sample preparation  $\times$  salt particle size interaction ( $P < 0.001$ , Table 2) observed. Specifically, the sample form (ground vs. unground) impacted CV in treatments containing coarse salt, but not those containing medium or fine salt. The main effect of mix time again did not impact CV (11.5, 11.0, and 10.0 for 3, 30, and 60 min. mix time, respectively). Treatments containing coarse salt again had greater CV ( $P < 0.0001$ ; 4.0, 7.7, and 17.4 for fine, medium and coarse, respectively). Groesbeck et al. (2004)<sup>4</sup> reported a lower CV after the particle size of the sample that contained coarse salt ( $\geq 730$  microns) was reduced from 700 compared with 400 micron salt. Sample preparation method also affected CV ( $P < 0.0001$ ), with ground samples having a more repeatable result (8.7% CV) than unground samples (13.0% CV).

In summary, an extended mix time up to 60 minutes did not lead to segregation of salt particles, even when coarse salt was used. However, if coarse salt is used in the feed manufacturing process, samples should be ground prior to mixer uniformity analysis.

**Table 1. Main effect of mix time on the uniformity of mix as determined by the coefficient of variation (CV) of feed (Experiment 1)<sup>1</sup>**

Item	Mix time, min							SEM	Probability, <i>P</i> =
	2	3	5	15	30	45	60		
CV, %	11.5	13.8	12.9	13.1	13.9	11.7	11.3	0.98	0.307

<sup>1</sup>Three particle sizes (350, 464 and 728 microns for fine, medium, and coarse, respectively) of salt were added to a swine grower diet and mixed in a 2.0 ft<sup>3</sup> double ribbon mixer for varying mix times to evaluate the role of salt particle size, mix time, and sample preparation on mix uniformity as measured by the chloride titrator strip method described by McCoy (2005).<sup>5</sup> Ten samples were collected from varying locations throughout the mixer and used to determine the coefficient of variation among samples. Lower values indicate less variation and a greater uniformity of mix across the mixer location.

**Table 2. Interactive effects of sample preparation and salt particle size on the coefficient of variation (CV) of feed mixed (Experiment 2)<sup>1</sup>**

Sample preparation	Salt particle size			SEM	Probability, <i>P</i> =
	Fine	Medium	Coarse		Sample preparation × particle size
Unground	8.1 <sup>a</sup>	9.0 <sup>a</sup>	21.8 <sup>c</sup>	0.88	0.0002
Ground	6.7 <sup>a</sup>	6.4 <sup>a</sup>	13.0 <sup>b</sup>		

<sup>abc</sup> Means with different superscripts are significantly different (*P* ≤ 0.05).

<sup>1</sup>Three particle sizes (350, 464, and 728 microns for fine, medium, and coarse, respectively) of salt were added to a swine grower diet and mixed in a 2.0 ft<sup>3</sup> double ribbon mixer for varying mix times to evaluate the role of salt particle size, mix time, and sample preparation on mix uniformity as measured by the chloride titrator strip method described by McCoy (2005).<sup>5</sup> Ten samples were collected from varying locations throughout the mixer and used to determine the coefficient of variation among samples. Lower values indicate less variation and a greater uniformity of mix across the mixer location.

## The Effect of Liquid Application Times, and Mixer Types with Different Wet Mix Times on Uniformity of Mix

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### Summary

Liquid addition systems are often designed to add liquid ingredients with the shortest application time in order to increase the batching capacity and efficiency of the mixing process. The quantity of liquid that is added into the mixer affects batch cycle time, particularly when there is a programmed “wet mix” time, or mixing time after liquid application has completed. Shorter application time of liquids typically produces a larger droplet size, which may lead to greater clumping tendencies in the feed and less uniformity of liquid incorporation. Two experiments were conducted to determine the effect of liquid application time and wet mix time on the uniformity of mix in different mixers. In both experiments, treatments were arranged in a  $2 \times 3$  factorial. Experiment 1 used a double ribbon mixer with 2 liquid application times (20 vs. 30 s) and 3 wet mix times (15, 30, and 45 s). Experiment 2 used a single shaft paddle mixer with 2 liquid addition times (15 vs. 30 s) and 3 wet mix times (15, 30 and 45 s). Ten samples were collected, and coefficient of variation (% CV) determined within those samples. Each treatment had 10 separate replicates. Experiment 1 indicated that wet mix time ( $P < 0.0001$ ), but not application time ( $P = 0.653$ ) or the interaction ( $P = 0.638$ ), impacted % CV in the double ribbon mixer. As wet mix time increased, % CV decreased in a quadratic manner ( $P = 0.02$ ; 37.2, 18.6, and 10.8% for 15, 30, and 45 s wet mix time, respectively). In Experiment 2, both wet mix time ( $P = 0.030$ ) and application time ( $P = 0.001$ ) impacted % CV, but not their interaction ( $P = 0.290$ ). A longer application time led to a better uniformity of mix ( $P < 0.05$ ; 13.5 vs. 9.8% CV for 15 vs. 30 s liquid application time), as did a longer wet mix time ( $P < 0.05$ ; 17.0, 9.8, and 8.2% CV for 10, 20, and 30 s wet mix time, respectively). These results suggest that extending liquid application times may be beneficial in some mixers, and underscore the importance of a sufficient wet mix time to maximize the uniformity of liquid incorporation.

Key words: liquid addition, wet mix, uniformity of mix

### Introduction

The number and quantity of liquid ingredients added to the mixer has increased during the last 10 years. The total quantity of liquids added to a mixer may affect batch cycle

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time, particularly if a constant wet mix time is utilized. The liquid application time varies based on the quantity of liquid applied and type of application system. Shorter application times typically produce a larger droplet size. When dry particles come in contact with a large droplet size, they have a greater propensity to clump, which may reduce the uniformity of the total mix. While some clumps were reduced by the shear force generated by the turning shaft of the mixer, this is dependent upon other factors, such as mixer type and wet mix time. Tekchandaney (2013)<sup>3</sup> concluded that a relationship exists between the shear degree and the mixer type, namely that double-paddle mixers have a slightly higher degree of shear, single-paddle and ribbon mixers have an average degree of shear, and the tumbling mixers have a low degree of shear. However, no data exist to evaluate the role of liquid application time or wet mix time in different types of mixers. The objective of these experiments was to determine the effect of liquid application time, and wet mix time on the uniformity of mix in two different types of mixers.

## Procedures

### *Materials and Method*

#### Experiment 1

Treatments were arranged in a 2 × 3 factorial of liquid application time (20 and 30 sec) and wet mix time (15, 30 and 45 s). Dry ingredients of a swine grower diet were mixed in a 2-ft<sup>3</sup> double ribbon mixer (model HP2SSS-0106, Fort Worth, Texas) for 15 s, followed by the addition of 18.4 oz of saline solution (2.27% per 60 lb batch) to the dry mixture in the mixer via hand-held sprayer (model 26329, Orscheln Farm & Home LLC) with 2 different application times by using different nozzles (TP11015 and TP11006, TeeJet Technologies). Feed was then mixed for 15, 30 and 45 s wet mix time after liquid application was complete. A total of 10 samples were obtained from 10 different locations throughout the mixer. There were 3 replicates per treatment.

#### Experiment 2

Treatments were arranged in a 2 × 3 factorial of liquid application time (15 and 30 sec) and wet mix time (15, 30, and 45 s). Dry ingredients of a swine grower diet were mixed in a 6-ft<sup>3</sup> paddle mixer (model 2014197-SS-S1, Bonner Springs, Kansas) for 15 s, followed by the addition of 61.3 oz of saline solution (2.27% per 200 lb batch) to the dry mixture with 2 different application times (15 and 30 sec) by using 2 and 4 hand-held sprayers (model 26329, Orscheln Farm & Home LLC) with a nozzle (model TP11020, TeeJet Technologies), respectively. Feed was then mixed for 15, 30 and 45 s wet mix time after liquid application was complete. A total of 10 samples were obtained from 10 different locations throughout the mixer. There were 3 replicates per treatment.

Chloride concentration in the collected samples from each experiment was determined via the Quantab<sup>®</sup> chloride titrator method (McCoy 2005).<sup>4</sup> A 10 g sample was weighed into a cup, and 90 g of hot distilled water (60°C) was added to the cup. The mixture was stirred for 30 s, allowed to rest for 60 s, and stirred for another 30 s. A folded filter paper was placed into the cup, and the Quantab<sup>®</sup> strip was inserted into the liquid at the

<sup>3</sup> Tekchandaney, J. K. 2013. Selection of solid blending equipment. Powder and Bulk Solids Exhibition & Conference, p. 1-5.

<sup>4</sup> McCoy, R. A. 2005. Chapter Appendix D: Mixer Testing. In Feed manufacturing technology V, 620-622. ed. E. K. Schofield and American Feed Industry Association, Arlington, VA: American Feed Industry Association.

bottom of the filter paper. The coefficient of variation (% CV) was calculated for each batch of feed. A lower CV was indicative of a greater uniformity of mix.

Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means adjustment for Bonferroni's multiple comparisons. Results were considered significant if  $P \leq 0.05$ .

## Results and Discussion

Experiment 1 indicated that wet mix time ( $P < 0.0001$ ) impacted % CV in the double ribbon mixer, but not application time ( $P = 0.653$ ; 22.7 vs. 21.8% CV for 20 vs. 30 s application time, respectively) or their interaction ( $P = 0.638$ ). As wet mix time increased, % CV decreased in a quadratic manner ( $P = 0.02$ ; Table 1). In Experiment 2, both application time ( $P = 0.030$ ) and wet mix time ( $P = 0.001$ ) impacted % CV, but not their interaction ( $P = 0.290$ ). A longer application time led to a better uniformity of mix ( $P < 0.05$ ; 13.5 vs. 9.8% CV for 15 vs. 30 s liquid application time), as did a longer wet mix time in a linear manner ( $P = 0.0004$ ; Table 2).

The results of these experiments indicate the importance of testing mixers at the time of installation, as specified by Current Good Manufacturing Practice requirements for medicated feed (FDA, 21 CFR part 225.30 (a), 2015).<sup>5</sup> The % CV of feed mixed with a ribbon mixer did not change when the liquid application time was decreased, while the % CV of the feed mixed with a paddle mixer increased when decreasing the liquid application time. The results demonstrated that mixer types and sizes may impact % CV. The results of these experiments also demonstrated that dry mix, liquid addition time, and wet mix time cannot be generically applied to mixers based on size and type.

In summary, the results of these experiments demonstrate that application time and wet mix time must be determined for each mixer type and size. In addition, while extended liquid application times may be beneficial, there must be a minimum fixed wet mix time after all of the liquids have been applied to the mixer.

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<sup>5</sup> U.S. Food and Drug Administration. 2015. Part 225 Current Good Manufacturing Practice For Medicated Feeds. U.S. Department of Health & Human Services.

**Table 1. Treatment main effect for wet mix time on the coefficient of variation (% CV) of feed mixed and sprayed with a 2.27% of saline solution in a 2-ft<sup>3</sup> double ribbon mixer (Experiment 1)**

Item	Wet mix time, sec			SEM	Probability, <i>P</i> =		
	15	30	45		Main effect	Linear	Quadratic
CV, %	37.21 <sup>a</sup>	18.63 <sup>b</sup>	10.79 <sup>c</sup>	1.70	<0.0001	<0.0001	0.0241

<sup>a-c</sup> Means with different superscripts are significantly different ( $P \leq 0.05$ ).

**Table 2. Treatment main effect for wet mix time on the coefficient of variation (% CV) of feed mixed and sprayed with a 2.27% of saline solution in a 2-ft<sup>3</sup> paddle mixer (Experiment 2)**

Item	Wet mix time, sec			SEM	Probability, <i>P</i> =		
	10	20	30		Main effect	Linear	Quadratic
CV, %	17.00 <sup>a</sup>	9.81 <sup>b</sup>	8.23 <sup>b</sup>	1.29	0.0009	0.0004	0.1004

<sup>a-b</sup> Means with different superscripts are significantly different ( $P \leq 0.05$ ).



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## Foreword

It is with great pleasure that we present the 2016 Swine Industry Day Report of Progress. This report contains updates and summaries of applied and basic research conducted at Kansas State University during the past year. We hope that the information will be of benefit as we attempt to meet the needs of the Kansas swine industry.

### **2016 Swine Day Report of Progress Editors**

Bob Goodband, Mike Tokach, Steve Dritz, Joel DeRouchey, and Jason Woodworth

## Standard Abbreviations

ADG	=	average daily gain	Mcal	=	megacalorie(s)
ADF	=	acid detergent fiber	ME	=	metabolizable energy
ADFI	=	average daily feed intake	mEq	=	milliequivalent(s)
AI	=	artificial insemination	min	=	minute(s)
avg	=	average	mg	=	milligram(s)
bu	=	bushel	mL	=	cc (cubic centimeters)
BW	=	body weight	mm	=	millimeter(s)
cm	=	centimeter(s)	mo	=	month(s)
CP	=	crude protein	MUFA	=	monounsaturated fatty acid
CV	=	coefficient of variation	N	=	nitrogen
cwt	=	100 lb	NE	=	net energy
d	=	day(s)	NDF	=	neutral detergent fiber
DE	=	digestible energy	NFE	=	nitrogen-free extract
DM	=	dry matter	ng	=	nanogram(s), .001 Fg
DMI	=	dry matter intake	no.	=	number
F/G	=	feed efficiency	NRC	=	National Research Council
ft	=	foot(feet)	ppb	=	parts per billion
ft <sup>2</sup>	=	square foot(feet)	ppm	=	parts per million
g	=	gram(s)	psi	=	pounds per square inch
µg	=	microgram(s), .001 mg	PUFA	=	polyunsaturated fatty acid
gal	=	gallon(s)	SD	=	standard deviation
GE	=	gross energy	sec	=	second(s)
h	=	hour(s)	SE	=	standard error
HCW	=	hot carcass weight	SEM	=	standard error of the mean
in	=	inch(es)	SEW	=	segregated early weaning
IU	=	international unit(s)	SFA	=	saturated fatty acid
kg	=	kilogram(s)	UFA	=	unsaturated fatty acid
kcal	=	kilocalorie(s)	wk	=	week(s)
kWh	=	kilowatt hour(s)	wt	=	weight(s)
lb	=	pound(s)	yr	=	year(s)

## K-State Vitamin and Trace Mineral Premixes

Diets listed in this report contain the following vitamin and trace mineral premixes unless otherwise specified.

- Trace mineral premix: Each pound of premix contains 10 g Mn, 33 g Fe, 33 g Zn, 5 g Cu, 90 mg I, and 90 mg Se.
- Vitamin premix: Each pound of premix contains 1,600,000 IU vitamin A, 400,000 IU vitamin D3, 8,000 mg vitamin E (dl- $\alpha$ -tocopherol acetate or 4,000 mg d- $\alpha$ -tocopherol acetate), 800 mg menadione, 1,500 mg riboflavin, 5,000 mg pantothenic acid, 15,000 mg niacin, and 7 mg vitamin B12.
- Sow add pack: Each pound of premix contains 100,000 mg choline, 40 mg biotin, 300 mg folic acid, 400 mg pyridoxine, 4,000 mg Vit E (dl- $\alpha$ -tocopherol acetate or 2,000 mg d- $\alpha$ -tocopherol acetate), 9,000 mg L-carnitine, and 36 mg Cr.

### *Note*

Some of the research reported here was carried out under special U.S. Food and Drug Administration (FDA) clearances that apply only to investigational uses at approved research institutions. Materials that require FDA clearances may be used in the field only at the levels and for the use specified in that clearance.

## Biological Variability and Chances of Error

Variability among individual animals in an experiment leads to problems in interpreting the results. Animals on treatment X may have higher average daily gains than those on treatment Y, but variability within treatments may indicate that the differences in production between X and Y were not the result of the treatment alone. Statistical analysis allows us to calculate the probability that such differences are from treatment rather than from chance.

In some of the articles herein, you will see the notation “ $P < 0.05$ .” That means the probability of the differences resulting from chance is less than 5%. If two averages are said to be “significantly different,” the probability is less than 5% that the difference is from chance, or the probability exceeds 95% that the difference resulted from the treatments applied.

Some papers report correlations or measures of the relationship between traits. The relationship may be positive (both traits tend to get larger or smaller together) or negative (as one trait gets larger, the other gets smaller). A perfect correlation is one (+1 or -1). If there is no relationship, the correlation is zero.

In other papers, you may see an average given as  $2.5 \pm 0.1$ . The 2.5 is the average; 0.1 is the “standard error.” The standard error is calculated to be 68% certain that the real average (with unlimited number of animals) would fall within one standard error from the average, in this case between 2.4 and 2.6.

Using many animals per treatment, replicating treatments several times, and using uniform animals increase the probability of finding real differences when they exist. Statistical analysis allows more valid interpretation of the results, regardless of the number of animals. In all the research reported herein, statistical analyses are included to increase the confidence you can place in the results.

## Index of Key Words

alternative	fat source	nursery feed
amino acid	feed additive	nursery pigs
amino acid ratio	feed manufacturing	particle size
antibiotic	feed matrix	PEDV
antimicrobial	finishing feed	pharmacological trace minerals
blending	fish meal	phosphorous
bone ash	flush	phytase
butyric acid	gilt training	phytogenics
calorie:lysine ratio	gluco-oligosaccharide	pigs
carbadox	glutamate	post-farrow maternal weight
carcass characteristics	glutamine	probiotic
chemical sanitation	group-housed gestating sows	protein source
chemical treatment	growing-finishing pig	reproduction
chlorine (Cl)	growth	salt
chromium propionate	growth performance	sample preparation
copper	HP 300	space allowance
copper amino acid-com- plex	isoleucine	source
crude protein	K-value	sow(s)
crude protein level	lactation	stocking density
diet complexity	<i>Lactobacillus plantarum</i>	superdose
dietary electrolyte balance	late finishing	swine
duration	level	tri-basic copper chloride
Elarom-F Plus	liquid addition	uniformity of mix
Elarom SES	lysine	valine
electrolyte balance	marketing	wet mix
electronic sow feeders	medium chain fatty acids	yeast
electronic sow feeding	Micro-Aid	zinc
enzymatically fermented soybean meal	mix time	zinc hydroxychloride
essential oil	Sodium (Na)	zinc sulfate
Evosure	net energy	
	nursery	

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