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Effects of Crystalline Amino Acid Concentrations With or Without Formaldehyde Treatment of Diets on Nursery Pig Growth Performance and Fecal Bacterial Concentration

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Abstract

A total of 1,235 nursery pigs (PIC 359 × 1050; initially 26.9 lb BW) were used in a 28-d study evaluating the effects of crystalline amino acid concentrations with or without formaldehyde treatment of diets on nursery pig growth performance, feed bacteria concentration, lysine content, and fecal microbial diversity. Sal CURB (Kemin Industries Inc., Des Moines, IA) is a commercial formaldehyde product that is commonly utilized in the poultry industry for *Salmonella* control in feed but has also been shown to reduce PEDV infectivity in swine diets.

Pigs were weaned at approximately 21 d, fed a common starter diet for 10 d, and allotted to pens based on BW in a completely randomized design. Experimental diets were fed in 2 phases (phase 1, d 0 to 12; and phase 2, 12 to 28 post-weaning) in meal form. Experimental treatments were arranged as a 2 × 2 + 1 factorial with main effects of formaldehyde (none vs. 0.30% in all phases) and crystalline AA concentration (low vs. high) plus a positive control. The positive control represented this current production system's formulated Lys requirement needed to maximize performance, whereas treatment diets were formulated at 80% of the positive control's lysine concentration. Feed bacterial concentration was determined by performing aerobic plate, Enterobacteriaceae, and total coliform counts on composited feed samples collected from each batch of feed manufactured at the feed mill and directly from feeders at the farm. Total, available, and free Lys analyses were conducted on composited feed samples collected from each phase of the study to determine Lys content. A composite fecal sample was collected from 3 randomly selected pigs per pen on d 28 for each treatment, DNA isolated, and each sample assessed for bacterial community analysis.

Overall, a significant crystalline AA × formaldehyde interaction ($P < 0.05$) was observed for ADFI and F/G. The interaction for ADFI was because added formaldehyde in high crystalline AA diets decreased feed intake; however, in low crystalline AA diets, ADFI was unchanged. For F/G, pigs had improved F/G in low crystalline AA diets without formaldehyde, but no difference was observed in high crystalline AA diets. Despite the interaction for ADFI and F/G, formaldehyde-treated diets reduced ($P < 0.05$) ADG, ADFI, and resulted in poorer F/G. Crystalline AA concentration did not impact performance. Added formaldehyde reduced or eliminated bacterial concentration of complete feed in phase 1 of the study. Formaldehyde reduced total and available Lys in both low and high crystalline AA diets, with a greater reduction occurring in low crystalline AA diets, but had no effect on free Lys. Added formaldehyde reduced ($P = 0.001$) Lactobacillaceae bacterial species, but increased ($P = 0.001$) Clostridiaceae bacterial species in fecal microbial samples. As expected, formaldehyde treatment reduced bacterial microflora of complete feeds. Overall, the level of crystalline AA did not impact performance while the nursery diet formaldehyde addition negatively influenced growth performance, AA utilization, and fecal microbial diversity.

Keywords

amino acids, formaldehyde, nursery, growth performance

Cover Page Footnote

Appreciation is expressed to Kemin Industries (Des Moines, IA) for technical and financial support of this experiment and Gene Gourley (Webster City, IA) for providing the animals, research facilities, and technical support.

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K-STATE Research and Extension

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Effects of Crystalline Amino Acid Concentrations With or Without Formaldehyde Treatment of Diets on Nursery Pig Growth Performance and Fecal Bacterial Concentration[1]

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Summary

A total of 1,235 nursery pigs (PIC 359 × 1050; initially 26.9 lb BW) were used in a 28-d study evaluating the effects of crystalline amino acid concentrations with or without formaldehyde treatment of diets on nursery pig growth performance, feed bacteria concentration, lysine content, and fecal microbial diversity. Sal CURB (Kemin Industries Inc., Des Moines, IA) is a commercial formaldehyde product that is commonly utilized in the poultry industry for *Salmonella* control in feed but has also been shown to reduce PEDV infectivity in swine diets.

Pigs were weaned at approximately 21 d, fed a common starter diet for 10 d, and allotted to pens based on BW in a completely randomized design. Experimental diets were fed in 2 phases (phase 1, d 0 to 12; and phase 2, 12 to 28 post-weaning) in meal form. Experimental treatments were arranged as a 2 × 2 + 1 factorial with main effects of formaldehyde (none vs. 0.30% in all phases) and crystalline AA concentration (low vs. high) plus a positive control. The positive control represented this current production system's formulated Lys requirement needed to maximize performance, whereas treatment diets were formulated at 80% of the positive control's lysine concentration. Feed bacterial concentration was determined by performing aerobic plate, Enterobacteriaceae, and total coliform counts on composited feed samples collected from each batch of feed manufactured at the feed mill and directly from feeders at the farm. Total, available, and free Lys analyses were conducted on composited feed samples collected from each phase of the study to determine Lys content. A composite fecal sample was col-

[1] Appreciation is expressed to Kemin Industries (Des Moines, IA) for technical and financial support of this experiment and Gene Gourley (Webster City, IA) for providing the animals, research facilities, and technical support.

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lected from 3 randomly selected pigs per pen on d 28 for each treatment, DNA isolated, and each sample assessed for bacterial community analysis.

Overall, a significant crystalline AA × formaldehyde interaction ($P < 0.05$) was observed for ADFI and F/G. The interaction for ADFI was because added formaldehyde in high crystalline AA diets decreased feed intake; however, in low crystalline AA diets, ADFI was unchanged. For F/G, pigs had improved F/G in low crystalline AA diets without formaldehyde, but no difference was observed in high crystalline AA diets. Despite the interaction for ADFI and F/G, formaldehyde-treated diets reduced ($P < 0.05$) ADG, ADFI, and resulted in poorer F/G. Crystalline AA concentration did not impact performance. Added formaldehyde reduced or eliminated bacterial concentration of complete feed in phase 1 of the study. Formaldehyde reduced total and available Lys in both low and high crystalline AA diets, with a greater reduction occurring in low crystalline AA diets, but had no effect on free Lys. Added formaldehyde reduced ($P = 0.001$) Lactobacillaceae bacterial species, but increased ($P = 0.001$) Clostridiaceae bacterial species in fecal microbial samples. As expected, formaldehyde treatment reduced bacterial microflora of complete feeds. Overall, the level of crystalline AA did not impact performance while the nursery diet formaldehyde addition negatively influenced growth performance, AA utilization, and fecal microbial diversity.

Introduction

Formaldehyde can be included in animal feed or ingredients to maintain complete feed and ingredients *Salmonella* negative for up to 21 d. Since the emergence of porcine epidemic diarrhea virus (PEDV) in the United States, formaldehyde products have received attention as a potential method to reduce the risk of PEDV transmission due to the ability of complete feed serving as a vector for the transmission of the disease.[4] To reduce this risk, research using formaldehyde to reduce PEDV infectivity in contaminated feed and ingredients has been successful.[5,6] However, formaldehyde is known to produce reactions with numerous groups of amino acid residues of proteins that can lead to the formation of methylol groups, Schiff-bases, and methylene bridges amongst these residues.[7] Thus, inclusion in diets may reduce the availability of dietary AA for pigs, which may influence growth performance and nutrient utilization. Limited research exists regarding the effects formaldehyde treatment of diets has on pig performance, and no data exist that measure the influence it has on fecal microbial concentrations. Therefore, the objective of this study was to evaluate the effects of dietary crystal-

[4] Dee, S., Clement, T., Schelkopf, A., Nerem, J., Knudsen, D., Christopher-Hennings, J. and E. Nelson 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. 10:176.doi: 10.1186/s12917-014-0176-9.

[5] Dee, S., C. Neill, T. Clement, J. Christopher-Hennings, and E. Nelson. 2015. An evaluation of a liquid antimicrobial (Sal CURB®) for reducing the risk of porcine epidemic diarrhea virus infection of naïve pigs during consumption of contaminated feed. BMC Veterinary Research. 10:220. doi 10.1186/s12917-014-0220-9.

[6] Cochrane, R. A., J. C. Woodworth, S. S. Dritz, A. R. Huss, C. R. Stark, R. A. Hesse, M. D. Tokach, J. F. Bai, and C. K. Jones. 2015. Evaluating chemical mitigation of Porcine Epidemic Diarrhea virus in swine feed and ingredients. Proc. ADSA-ASAS 2015 Midwest Meeting.

[7] Metz, B., G.F. Kersten, P. Hoogerhout, H.F. Brugghe, H.A. Timmermans, A.D. De Jong, H. Meiring, J. ten Hove, W.E. Hennink, D.J. Crommelin, and W. Jiskoot. 2004. Identification of formaldehyde-induced modifications in proteins reactions with model peptides. J. Bio. Chem. 279:6235-6243. doi:10.1074/jbc.M310752200.

line AA concentrations with or without formaldehyde treatment of diets on nursery pig growth performance, feed bacteria concentration, and fecal microbial diversity.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The trial was conducted in a tunnel-ventilated commercial grow-finish barn in central Iowa (Gourley Bros. Inc., Webster City, IA). Each pen (18.5 × 8 ft) had slatted flooring, one 4-hole self-feeder, and a pan waterer to provide ad libitum access to feed and water.

A total of 1,235 nursery pigs (PIC 359 × 1050; initially 26.9 lb BW) were used in a 28-d study with 19 to 22 pigs per pen and 12 replications per treatment. Pigs were weaned at approximately 21 d of age, fed a common starter diet for 10 d, and allotted to 1 of 5 dietary treatments based on average BW and location within barn in a completely randomized block design.

The treatments were arranged in a 2 × 2 + 1 factorial with main effects of formaldehyde (none vs. 0.30% Sal CURB; Kemin Industries Inc., Des Moines, IA) and crystalline AA inclusion (low vs. high) plus a positive control diet. Sal CURB is a premix of 37% aqueous formaldehyde and propionic acid. All treatment diets were formulated to be 80% of the standardized ileal digestible (SID) Lys of that contained in the positive control, which was also 90 to 95% of the SID Lys requirement according to NRC.[8] A positive control was used in the experiment to represent diets that met the assumed SID Lys requirement for maximum growth performance in this system. The treatment ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1).

All diets were corn-soybean meal-based and were formulated in 2 phases. Diets were fed in meal form and were prepared at a commercial feed mill (Altoona, IA). Formaldehyde inclusion and application methods were conducted according to manufacturers' recommendations, with inclusion occurring in the mixer. Diets in each phase were collected from the mill and 6 randomly selected feeders, pooled within collection location, and submitted for analysis of DM, CP, Ca, P, propionic acid, and Lys content; specifically, total Lys, free Lys, and available Lys (Tables 2 and 4). Propionic acid was analyzed according to manufacturer's procedures and was analyzed to confirm correct inclusion rates of Sal CURB to treatment diets.

Feed bacterial concentration was tested for samples collected during manufacturing and from the farm using 3M Petrifilm plates (3M Microbiology, St. Paul, MN) with each of these plates selecting for: total coliforms (TC), aerobic plate counts (APC), or Enterobacteriaceae (EB). A plate reader was used to enumerate each plate for specific ranges, colony morphology, gas production, and acidification. Colony counts were expressed as colony forming units per gram of feed sample (cfu/g) and bacterial counts were expressed as an average of 2 separate runs processed in duplicate with a different feed sample.

[8] NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press., Washington D.C.

Fecal samples were collected into individual Whirl-Pak bags via rectal massage from 6 randomly pigs per pen on d 28. Samples were stored at 4°C and then transported to Kansas State University where d 28 samples were pooled within pen, for a final total of 12 samples per treatment. Samples were stored at -80°C until they were transported to the University of Nebraska-Lincoln for bacterial isolation and community analysis. Fecal DNA from the pooled samples were isolated from 100 mg of each sample and PCR analysis was performed to amplify the 16S rRNA gene specific to bacterial communities. The amplified gene was then sequenced and subjected to bacterial community analysis.

Growth data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Pre-planned contrasts were utilized to compare the interactive and main effects of formaldehyde and crystalline AA inclusion, and the positive control vs. the other treatments. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

Bacterial community data were analyzed as a completely randomized block design using the PROC GLIMMIX procedure of SAS and the responses were presented as least-squares means (± SEM). Additionally, OTU abundances at family level in the bacterial communities were analyzed using the GLIMMIX procedure of SAS. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Results of the diet analysis closely matched those of formulated levels (Table 2). Propionic acid analysis of diets, used as an indicator of formaldehyde concentration as well, confirmed that targeted concentrations were at the correct levels in respective dietary treatments.

Analysis of both total Lys and available Lys in the positive control and treatment diets revealed lower levels than the formulated values (Table 3). In phase 1, the low crystalline AA formulated diets total and available Lys were reduced 8.7 and 10.4% when diets were treated with formaldehyde, respectively. In high crystalline AA diets, formaldehyde inclusion marginally reduced total and available Lys by 3.2% each. In phase 2, formaldehyde addition in low crystalline AA formulated diets reduced total and available Lys by 12.6 and 13.1%, respectively. Added formaldehyde in high crystalline AA formulated diets had little to no effect on total Lys with a reduction of 0.93 and 0.91%, respectively. Formaldehyde addition had no observed effect on free Lys, which can be considered an indicator of the amount of crystalline AA added to the diets.

For diet bacterial concentrations, as anticipated, formaldehyde treatment reduced the bacterial concentration of samples collected at both the feed mill and the farm compared to diets not treated with formaldehyde (Table 4). However, in phase 2 feed mill and the farm samples, bacterial loads for either non-treated or treated diets with Sal CURB were similar regardless of crystalline amino acid inclusion, except for the high crystalline treatments collected at the feed mill where formaldehyde treatment did reduce bacterial load.

From d 0 to 12, there was no evidence ($P > 0.10$) for a crystalline AA concentration × formaldehyde interactions or crystalline AA concentration main effects (Table 5). Pigs fed diets containing formaldehyde had poorer ($P = 0.001$) ADG and F/G compared to pigs fed diets that did not contain formaldehyde. Pigs fed the control diet had better ($P = 0.05$) ADG and F/G compared to pigs fed the other diets containing reduced Lys.

From d 12 to 28, a crystalline AA × formaldehyde interaction ($P < 0.05$) was observed for ADFI and F/G. This interaction for feed intake was a result of pigs fed high crystalline AA diets treated with formaldehyde having lower ADFI compared to the non-treated feed, while in the low crystalline AA diets formaldehyde treatment had no effect on ADFI. The F/G interaction was observed because pigs fed low crystalline AA diets without the inclusion of formaldehyde resulted in better F/G than pigs fed diets with formaldehyde; however, the inverse was observed in high crystalline AA diets. A tendency ($P = 0.073$) for a crystalline AA × formaldehyde interaction was observed for ADG, with pigs fed the low crystalline AA diets having a more dramatic decrease in ADG when formaldehyde was included compared to the high crystalline AA diets. Pigs fed formaldehyde-treated feed had reduced ($P < 0.05$) ADG and d 28 BW, they also tended ($P = 0.052$) to have reduced ADFI compared to pigs fed non-formaldehyde-treated diets. Pigs fed the control diet had better ($P < 0.05$) ADG and F/G compared to pigs fed the other diets containing reduced Lys. There was no evidence of difference between diets containing low and high levels of crystalline AA for any growth criteria measured.

Overall (d 0 to 28), a significant crystalline AA × formaldehyde interaction ($P < 0.05$) was observed for ADFI and F/G. The interaction for ADFI occurred because pigs fed diets with high crystalline AA inclusions and formaldehyde treatment had poorer ADFI compared to pigs fed diets without formaldehyde, but in the low crystalline AA diets, ADFI was the same. The interaction for F/G was observed because pigs fed low crystalline AA diets without formaldehyde had better F/G than with the formaldehyde treatment, but pigs fed high levels of crystalline AA had similar F/G regardless of formaldehyde inclusion. Despite the interaction, the application of formaldehyde to diets resulted in reduced ($P < 0.05$) ADG, ADFI, and ending BW and poorer F/G compared to diets without the application of formaldehyde. Pigs fed the control diet had improved ($P < 0.05$) ADG, ending BW, and F/G compared to those fed other diets containing reduced Lys. There was no evidence of difference between diets containing low and high levels of crystalline AA for any response criteria measured throughout the trial.

For bacterial community abundance, no evidence of a difference ($P > 0.10$) in bacterial abundances amongst the dietary treatments for Methanobacteriaceae, Prevotellaceae, Lachnospiraceae, or Spirochaetaceae (Table 6). A significant crystalline AA × formaldehyde interaction ($P = 0.003$) was observed for Streptococcaceae abundances in the bacterial community of the gut, because pigs fed low crystalline AA diets had a more dramatic reduction in abundance when treated with formaldehyde compared to the high crystalline AA diets. The treatment of diets with formaldehyde decreased ($P < 0.05$) bacterial abundance for Paraprevotellaceae and Lactobacillaceae species, while formaldehyde treatment increased ($P < 0.05$) Clostridiaceae and Erysipelotrichaceae species within the bacterial community of the gut. Pigs fed formaldehyde-treated

diets tended ($P = 0.074$) to have lower percentages of S24-7 bacteria species than pigs fed non-formaldehyde treated diets. Pigs fed low crystalline AA diets had increased ($P < 0.05$) abundance of Paraprevotellaceae, Lactobacilliaceae, Ruminococcaceae, and Veillonellaceae bacterial species compared to high crystalline AA diets. Pigs fed high crystalline AA diets had increased ($P = 0.007$) Clostridiaceae and tended ($P = 0.080$) to have increased Erysipelotrichaceae bacterial species compared to pigs fed low crystalline AA diets. Treatment diets fed to lower lysine levels than the control had increased ($P = 0.009$) Clostridiaceae bacterial species, while Paraprevotellaceae species tended ($P = 0.091$) to be lower in these diets compared to the positive control.

These data provide evidence that in late-nursery pigs the inclusion of formaldehyde in complete feeds has a negative impact on ADG, ADFI, F/G, and ending BW, when diets are fed below the Lys requirement of the pigs. Furthermore, it can be observed that inclusion of formaldehyde in complete nursery diets reduced the amount of total and available Lys within the diet, which suggest formaldehyde is affecting AA availability of the diet. Formaldehyde treatment of complete feeds also negatively impacts the gut microflora of late nursery pigs. This can be observed in the decrease of lactic acid bacterial species, specifically Lactobacillaceae that has the potential to improve gastrointestinal function. However, formaldehyde treatment increased Clostridiaceae bacterial species that can lead to enteric disruptions and promote proliferation of enteric disease.

These results suggest that the level of crystalline AA in the diets did not impact performance. In summary, formaldehyde treatment of feed reduced bacterial concentration within complete diets and affected fecal bacterial abundance. Also, these results suggest that formaldehyde is effective at reducing pathogen load within the feed, but formulation adjustments should be considered to reduce the negative impact on performance due to decreased AA availability.

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

	Phase 1			Phase 2		
Ingredient, %	Control[2]	Low crystalline AA[3]	High crystalline AA[3]	Control[2]	Low crystalline AA[3]	High crystalline AA[3]
Corn	45.61	46.10	56.19	43.48	43.90	58.70
Soybean meal (46.5% CP)	47.64	37.60	28.21	30.36	30.33	16.58
Corn DDGS, 6-9% oil[4]	10.00	10.00	10.00	20.00	20.00	20.00
Choice white grease	3.20	3.25	2.00	3.40	3.45	1.65
Limestone	1.08	1.08	1.13	1.15	1.15	1.25
Monocalcium phosphate, 21% P	0.80	0.80	0.85	0.40	0.40	0.47
Sodium chloride	0.46	0.46	0.46	0.41	0.41	0.41
L-Lys-HCL	0.41	0.05	0.34	0.33	---	0.43
L-Thr	0.13	---	0.13	0.08	---	0.10
L-Trp	---	---	0.01	---	---	0.03
Phytase[5]	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral and vitamin premix	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin E[6]	0.05	0.05	0.05	0.05	0.05	0.05
Zinc oxide	0.15	0.15	0.15	0.06	0.06	0.06
Copper sulfate	0.13	0.13	0.13	0.13	0.13	0.13
Medication[7]	0.20	0.20	0.20	---	---	---
Formaldehyde[8]	---	---	---	---	---	---
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
Standardized ileal digestible (SID) AA, %						
Lys	1.45	1.17	1.17	1.25	0.99	0.99
Met:Lys	38	48	44	38	49	43
Met and Cys:Lys	61	75	68	63	80	68
Thr:Lys	61	65	65	63	72	63
Trp:Lys	18.1	22.4	18.8	18.5	23.4	18.8
Val:Lys	67	84	71	75	94	72
Total Lys, %	1.64	1.36	1.33	1.45	1.19	1.15
ME, kcal/lb	1,555	1,554	1,532	1,568	1,567	1,535
CP, %	25.1	24.7	21.4	24.0	23.6	18.8
Ca, %	0.66	0.66	0.66	0.61	0.61	0.61
P, %	0.62	0.62	0.59	0.53	0.54	0.49
Available P, %	0.40	0.40	0.40	0.35	0.35	0.35

[1] Phase 1 diets fed from ~26.9 to 38.7 lb BW and phase 2 diets from ~38.7 to 60.5 lb BW.
[2] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).
[3] Treatment diets were formulated to 80% of the control diet and contained low or high levels of crystalline AA.
[4] Dried distillers grains with solubles.
[5] Optiphos 2000 (Huvepharma LLC., Sofia, Bulgaria), provided 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.
[6] 20,000 IU.
[7] CTC, Zoetis Services, LLC., Florham Park, NJ.
[8] Kemin Industries, Inc., Des Moines, IA. Sal CURB added 0.30% of the diet in all phases (d 0-28) according to manufacturer's recommendations.

Table 2. Chemical analysis of experimental diets, %[1,2]

		Low crystalline		High crystalline	
	Control[3]	No formaldehyde	Formaldehyde[4]	No formaldehyde	Formaldehyde[4]
Phase 1 diets					
DM	91.0	91.0	89.9	90.3	90.0
CP	25.3	25.9	24.1	21.7	21.0
Ca	0.68	0.77	0.81	0.76	0.98
P	0.62	0.69	0.60	0.60	0.65
Propionic acid, ppm[5]	<LOQ[6]	<LOQ	295	<LOQ	300
Phase 2 Diets					
DM	90.2	90.4	89.9	90.2	89.6
CP	23.8	23.7	22.7	18.9	19.6
Ca	0.53	0.71	0.63	0.73	0.52
P	0.58	0.62	0.55	0.60	0.57
Propionic acid, ppm	<LOQ	<LOQ	305	<LOQ	300

[1] Phase 1 diets fed from ~26.9 to 38.7 lb BW and phase 2 diets from ~38.7 to 60.5 lb BW.

[2] Complete diet samples were obtained from each dietary treatment during manufacturing and from the farm feeder. Samples of diets were pooled and then submitted for analysis of DM, CP, Ca, and P (Ward Laboratories, Inc., Kearney, NE).

[3] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).

[4] Sal CURB (Kemin Industries, Inc., Des Moines, IA) added at 6.0 lb/ton in all phases (d 0-28).

[5] Propionic acid testing conducted according to Kemin Industries, Inc. sampling methods.

[6] Level of quantification.

Table 3. Effect of formaldehyde-treated diets and crystalline amino acid level on dietary lysine content, %[1,2]

		Low crystalline		High crystalline	
	Control[3]	No formaldehyde	Formaldehyde[4]	No formaldehyde	Formaldehyde[4]
Phase 1					
Calculated					
Total Lys	1.64	1.36	1.36	1.33	1.33
Free Lys	0.41	0.05	0.05	0.34	0.34
Analyzed					
Total Lys	1.59	1.32	1.21	1.28	1.24
Available Lys	1.56	1.32	1.19	1.29	1.25
Free Lys	0.30	0.06	0.06	0.25	0.26
Phase 2					
Calculated					
Total Lys	1.45	1.19	1.19	1.15	1.15
Free Lys	0.33	ND[5]	ND	0.43	0.43
Analyzed					
Total Lys	1.38	1.18	1.04	1.11	1.10
Available Lys	1.37	1.14	1.00	1.08	1.07
Free Lys	0.23	0.02	0.02	0.27	0.33

[1] Phase 1 diets fed from ~26.9 to 38.7 lb BW and phase 2 diets from ~38.7 to 60.5 lb BW.

[2] Complete diet samples were obtained from each dietary treatment during manufacturing and from the farm feeder. Samples of diets were pooled and then submitted for analysis of total lysine, available lysine, and free lysine (Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO). Values represent average of duplicate analyses on pooled samples.

[3] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).

[4] Sal-CURB, Kemin Industries Inc., Des Moines, IA.

[5] Indicates no detection.

Table 4. Effect of formaldehyde-treated diets and crystalline amino acid level on complete feed bacterial concentration[1,2,3]

		Low crystalline[5]		High crystalline[5]	
	Control[4]	No formaldehyde	Formaldehyde[6]	No formaldehyde	Formaldehyde[6]
Phase 1 feed mill[7]					
Aerobic plate count	1.7×10^5	5.3×10^4	6.1×10^4	7.9×10^4	5×10^3
Enterobacteriaceae count	3.2×10^3	1.5×10^3	0	4.6×10^3	0
Total coliform count	3.5×10^3	1.2×10^4	0	9.0×10^3	0
Phase 1 Farm[8]					
Aerobic plate count	2.2×10^5	8.6×10^4	8.0×10^4	1.3×10^5	8×10^3
Enterobacteriaceae count	6.7×10^3	2.9×10^3	0	3.4×10^4	0
Total coliform count	5.9×10^4	1.5×10^4	0	6.5×10^4	0
Phase 2 feed mill[7]					
Aerobic plate count	2.6×10^5	4.5×10^4	2.3×10^5	4.8×10^4	3.8×10^4
Enterobacteriaceae count	2.0×10^4	5.5×10^3	1.0×10^4	1.0×10^4	0
Total coliform count	4.2×10^4	5.5×10^3	1.5×10^4	4.4×10^4	0
Phase 2 Farm[8]					
Aerobic plate count	1.1×10^6	4.7×10^5	3.5×10^4	1.3×10^5	4.6×10^5
Enterobacteriaceae count	7.0×10^4	2.8×10^4	3.1×10^3	2.7×10^4	6.9×10^4
Total coliform count	3.6×10^5	5.5×10^4	3.7×10^4	4.9×10^4	2.5×10^5

[1] Phase 1 diets fed from ~26.9 to 38.7 lb BW and phase 2 diets from ~38.7 to 60.5 lb BW.

[2] Complete feed samples from each dietary treatment and phase were collected during manufacturing and from the farm for enumeration of feed bacterial concentration (Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS).

[3] Feed bacterial concentrations are expressed as colony forming units per gram of feed sample (cfu/g).

[4] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).

[5] Treatment diets were formulated to 80% of the control diet and contained low or high levels of crystalline AA.

[6] Sal-CURB, Kemin Industries Inc., Des Moines, IA.

[7] Indicates feed samples were collected directly from each individual batch of feed for each dietary treatment in each phase during manufacturing. Five equally spaced sub-samples were collected by passing sterile Whirl-Pak through stream of lot during manufacturing and pooled to create one composite sample for each dietary treatment in each phase to represent feed mill sample.

[8] Indicates feed samples were collected from 6 randomly chosen feeders from each dietary treatment in each phase. The 6 sub-samples were then pooled into one composite sample for each dietary treatment in each phase to represent farm sample.

Table 5. Effect of formaldehyde-treated diets and crystalline amino acid level on nursery pig performance[1]

		Low crystalline[3]		High crystalline[3]			Probability $P<$			
	Control[2]	No formaldehyde	Formaldehyde[4]	No formaldehyde	Formaldehyde[4]	SEM	Control vs. others	Crys AA × formaldehyde	Low crys AA vs. high crys AA	Formaldehyde
d 0 to 12										
ADG, lb	1.08	0.98	0.94	1.01	0.91	0.02	0.001	0.103	0.910	0.001
ADFI, lb	1.53	1.52	1.52	1.57	1.50	0.03	0.584	0.105	0.402	0.116
F/G	1.43	1.56	1.61	1.55	1.67	0.02	0.001	0.140	0.209	0.001
d 12 to 28										
ADG, lb	1.51	1.40	1.28	1.34	1.32	0.02	0.001	0.073	0.526	0.009
ADFI, lb	2.50[a,b]	2.44[b,c]	2.45[a,b,c]	2.53[a]	2.40[c]	0.04	0.177	0.023	0.594	0.052
F/G	1.65[a]	1.79[b]	1.92[c]	1.90[c]	1.82[b]	0.02	0.001	0.001	0.915	0.119
d 0 to 28										
ADG, lb	1.32	1.20	1.13	1.20	1.14	0.02	0.001	0.757	0.637	0.001
ADFI, lb	2.08[a,b]	2.05[b,c]	2.05[b,c]	2.12[a]	2.01[c]	0.03	0.235	0.020	0.526	0.040
F/G	1.57[a]	1.71[b]	1.81[d]	1.77[c]	1.77[c]	0.01	0.001	0.001	0.478	0.001
BW, lb										
d 0	26.8	26.9	27.1	26.8	26.9	0.264	0.521	0.278	0.142	0.139
d 12	39.8	38.6	38.4	40.0	37.7	0.397	0.002	0.055	0.626	0.009
d 28	64.1	60.3	59.0	60.5	58.9	0.611	0.001	0.713	0.931	0.001

[a,b,c,d] Means within same row with different superscripts differ ($P < 0.05$).

[1] A total of 1,235 pigs (PIC 359 × PIC 1050, initially 26.9 ± 0.02 lb) were used in a 2-phase nursery study with 19 to 22 pigs per pen and 12 replications per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 10 d post-weaning, and then fed experimental diets.

[2] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).

[3] Treatment diets were formulated to 80% of the control diet and contained low or high levels of crystalline AA.

[4] Sal-CURB, Kemin Industries Inc., Des Moines, IA.

Table 6. Effect of formaldehyde-treated diets and crystalline amino acid level on fecal bacterial abundances at phylum level[1,2]

		Low crystalline[4]		High crystalline[4]			Probability $P <$			
	Control[3]	No formaldehyde	Formaldehyde[5]	No formaldehyde	Formaldehyde[5]	SEM	Control vs. others	Crys AA × formaldehyde	Low crys AA vs. high crys AA	Formaldehyde
Abundances, %[6]										
Methanobacteriaceae	3.71	4.74	5.83	4.98	5.11	1.08	0.224	0.661	0.824	0.578
Prevotellaceae	10.5	11.9	10.6	8.57	9.07	1.56	0.796	0.568	0.129	0.802
S24-7	3.38	4.22	3.75	4.41	2.94	0.53	0.458	0.352	0.557	0.074
Paraprevotellaceae	1.16	1.33	0.69	0.81	0.48	0.17	0.091	0.371	0.041	0.008
Lactobacillaceae	17.3	11.9	0.60	9.90	1.04	1.88	0.682	0.532	0.001	0.001
Clostridiaceae	19.0	19.2	27.5	25.9	35.5	2.65	0.009	0.796	0.007	0.001
Streptococcaceae	4.52	6.19	0.02	3.30	0.31	0.53	0.001	0.003	0.011	0.001
Lachnospiraceae	8.27	7.95	10.6	9.50	10.2	1.21	0.338	0.440	0.639	0.169
Ruminococcaceae	11.7	11.1	13.2	10.0	10.6	0.88	0.661	0.410	0.038	0.136
Veillonellaceae	1.53	1.83	2.03	1.55	0.98	0.25	0.790	0.126	0.010	0.471
Erysipelotrichaceae	2.12	1.97	2.59	2.51	3.19	0.33	0.210	0.918	0.08	0.047
Spirochaetaceae	1.04	0.78	0.67	0.59	0.51	0.30	0.211	0.953	0.550	0.760

[1] A total of 1,235 pigs (PIC 359 × PIC 1050, initially 26.9 ± 0.02 lb) were used in a 2-phase nursery study with 19 to 22 pigs per pen and 12 replications per treatment. Pigs were weaned at approximately 21 d, fed a common starter diet for 10 d post-weaning, and then fed experimental diets.

[2] 3 random fecal samples were collected per pen on d 28 of the trial and pooled to form 1 composite sample for each pen on each dietary treatment, DNA was isolated, and each composited sample was assessed.

[3] Control diets were formulated to exceed the SID Lys requirement (NRC, 2012).

[4] Treatment diets were formulated to 80% of the control diet and contained low or high levels of crystalline AA.

[5] Sal-CURB, Kemin Industries Inc., Des Moines, IA.

[6] Bacterial species that composed at least 1% of total bacterial population in an individual treatment.