

## Effects of a Bacillus-Based Feed Additive in Nursery Pig Diets Containing Low or High Levels of Soybean Meal

*Ty H. Kim, Grady A. Privett, Pau Salgado-Lopez, Mike D. Tokach, Joel M. DeRouchey, Jason C. Woodworth, Robert D. Goodband, Katelyn N. Gaffield, Thomas E. Weber,<sup>1</sup> Kale Causemaker,<sup>1</sup> Brooke Anderson,<sup>1</sup> Dwight J. Shawk,<sup>2</sup> Noah C. Gainey,<sup>2</sup> Veronica Polniak,<sup>2</sup> and Jordan T. Gebhardt<sup>3</sup>*

### Summary

A total of 4,320 nursery pigs (PIC 337 × 1050, initially 14.7 ± 0.21 lb) were used in a 42-d study to determine the effects of a bacillus-based feed additive on growth performance and fecal dry matter (DM) in nursery pig diets with low or high levels of soybean meal (SBM). At approximately 21 d of age, pigs were weaned, blocked by initial BW and allotted to one of four dietary treatments in a randomized complete block design with 20 replications per treatment. Diets were formulated in three phases and fed from d 0 to 7, 7 to 21, and 21 to 42, respectively. The four treatments were arranged in a 2 × 2 factorial with main effects of bacillus-based feed additive (none or 10<sup>6</sup> colony-forming units (CFU)/g of complete feed; Argis, Kent Nutrition Group, Muscatine, IA) and SBM level (low or high). On approximately d 14 (range of 9 to 20 days post placement) of the study, fecal samples were collected from three pigs per feeder to determine fecal DM. From d 0 to 7, an SBM × bacillus interaction was observed ( $P < 0.05$ ) for ADFI, where pigs fed high SBM diets had decreased ADFI compared to pigs fed low SBM when bacillus was not in the diet; however, when bacillus was in the diet, SBM level did not affect ADFI. Additionally, pigs fed high SBM had decreased ( $P < 0.05$ ) ADG and poorer F/G compared to pigs fed low SBM. From d 7 to 21, the SBM × bacillus interaction was observed ( $P < 0.05$ ) for ADG and F/G, where pigs fed high SBM had increased ADG and improved F/G compared to pigs fed low SBM when bacillus was not in the diet; however, when bacillus was in the diet, SBM level did not affect ADG, and there was a smaller improvement in F/G in pigs fed high SBM compared to those fed low SBM. Pigs fed high SBM had decreased ( $P < 0.05$ ) ADFI compared to pigs fed low SBM. From d 21 to 42, there was a tendency ( $P = 0.076$ ) for pigs fed bacillus to have higher ADG compared to pigs not fed bacillus. Overall, the SBM × bacillus interaction was observed ( $P < 0.05$ ) for F/G, where pigs fed high SBM had improved F/G compared to pigs fed low SBM when bacillus was not in the diet; however, when bacillus was in the diet, SBM level did not affect F/G.

<sup>1</sup> Kent Nutrition Group, Muscatine, IA.

<sup>2</sup> Hord Family Farms, Bucyrus, OH.

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

For fecal DM, pigs fed high SBM had decreased ( $P < 0.05$ ) fecal DM compared to pigs fed low SBM. The SBM  $\times$  bacillus interaction was observed ( $P < 0.05$ ) for total removals and mortality, where pigs fed high SBM had increased total removals and mortality compared to pigs fed low SBM when bacillus was not in the diet; however, when bacillus was in the diet, SBM level did not affect total removals and mortality. In summary, high SBM had negative effects on phase 1 growth, fecal DM, and removals and mortality, but high SBM improved phase 2 ADG and phase 2 and 3 F/G. When bacillus was not in the diet, high SBM increased removals and mortality; however, when bacillus was in the diet, SBM level did not affect removals and mortality.

## Introduction

Swine diets often contain soybean meal (SBM) as the primary plant-based protein source. However, SBM inclusion level is limited in the nursery period due to antinutritional factors (ANFs), such as trypsin inhibitors, non-starch polysaccharides (NSPs), nondigestible oligosaccharides, and the antigenic proteins glycinin and beta-conglycinin.<sup>4,5,6</sup> These ANFs can cause poor growth performance and post-weaning diarrhea in early nursery pigs.

Bacillus-based feed additives are a class of direct-fed microbials (DFMs). These DFMs can improve gut health by increasing short-chain fatty acid production and reducing pathogenic bacteria, which can lead to improvements in growth and nutrient digestibility.<sup>7</sup> As a result, it is hypothesized that bacillus-based feed additives could improve nutrient digestibility and decrease negative effects of ANFs in diets containing high SBM levels. Therefore, the objective of this study was to determine the effect of a bacillus-based feed additive on growth performance and fecal DM in nursery pig diets containing low or high levels of SBM. A second objective was to determine if pigs fed the bacillus-based feed additive in diets containing a high level of SBM could exhibit similar growth performance and fecal DM as pigs fed a low level of SBM.

## Materials and Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at a commercial research facility in north central Ohio (Bucyrus, OH). A total of 160 pens were used with 80 double-sided, five-hole stainless steel fence-line feeders to provide *ad libitum* access to feed. Each feeder supplied feed to two adjacent pens, with feeder serving as the experimental unit. For each feeder, one pen contained 27 gilts, and one pen contained 27 barrows. Each pen was also equipped with a cup waterer to provide *ad libitum* access to water.

<sup>4</sup> Choct, M., Y. Dersjant-Li, J. McLeish, and M. Peisker. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive, and anti-nutritive effects in pigs and poultry. *Asian-Aust. J. Anim. Sci.* 23:1386-1398. doi:10.5713/ajas.2010.90222.

<sup>5</sup> Woyengo, T. A., E. Beltranena, and R. T. Zijlstra. 2017. Effect of anti-nutritional factors of oilseed co-products on feed intake of pigs and poultry. *Animal Feed Sci. Technol.* 233:76-86. doi:10.1016/j.anifeeds.2016.05.006.

<sup>6</sup> Ruckman, L. A., A. L. Petry, S. A. Gould, B. J. Kerr, and J. F. Patience. 2020. The effects of enzymatically treated soybean meal on growth performance and intestinal structure, barrier integrity, inflammation, oxidative status, and volatile fatty acid production of nursery pigs. *Transl. Anim. Sci.* 4:1-16. doi:10.1093/tas/txaa170.

<sup>7</sup> Liao, S. F. and M. Nyachoti. 2017. Using probiotics to improve swine gut health and nutrient utilization. *Anim. Nutr.* 3:331-343. doi:10.1016/j.aninu.2017.06.007.

A total of 4,320 nursery pigs (PIC 337 × 1050) were weaned at approximately 21 d of age and loaded into four rooms in a barn across a 12-d period. Pigs were blocked by sow source, entry date, and initial BW (initially  $14.7 \pm 0.21$  lb) and allotted to one of four dietary treatments in a randomized complete block design with 20 replications per treatment. The four treatments were arranged in a  $2 \times 2$  factorial with main effects of bacillus-based feed additive (none or  $10^6$  colony-forming units (CFU)/g of complete feed; Argis, Kent Nutrition Group, Muscatine, IA) and SBM level (low or high).

Treatment diets were formulated in three dietary phases and fed from d 0 to 7, 7 to 21, and 21 to 42, respectively (Table 2). Phase 1 diets were manufactured at Premier Feeds (Urbana, OH) and were in pellet form. Phase 2 and 3 diets were manufactured at the Hord Farms Feed Mill (Bucyrus, OH) and were in mash form. Daily feed additions to each pen were accomplished using an automated feeding system (DryExact Pro; Big Dutchman North America, Holland, MI) that recorded feed deliveries for individual pens.

Prior to diet formulation, representative samples of corn and SBM were submitted for complete proximate and amino acid analysis in duplicate (University of Missouri Agricultural Experiment Station Chemical Laboratory; Table 1). Analyzed values were used in diet formulation. Nutrient loading values and standardized ileal digestibility coefficients from NRC (2012) were used for all nutrients where analytical values were not available. Complete diet samples were collected from each feeder and were pooled, ground to reduce particle size, and stored at  $-4^{\circ}\text{F}$  until they were submitted for complete proximate analysis in duplicate (University of Missouri Agricultural Experiment Station Chemical Laboratory, Columbia, MO). Pigs were weighed and feed disappearance was measured every 7 d to determine ADG, ADFI, and F/G.

Fecal samples were collected from three pigs per feeder (60 pigs per treatment) on the same day on approximately d 14 (range of 9 to 20 d post-placement) of the study. After collection, fecal samples were dried at  $131^{\circ}\text{F}$  in a forced air oven for 48 h, and fecal DM was determined as the ratio of dried to wet fecal weight.

### *Statistical analysis*

Experimental data were analyzed as a randomized complete block design with feeder as the experimental unit and weight block as a random effect. The main effects of SBM level and bacillus, as well as their interactions, were tested. Total removals and mortality data were analyzed assuming a binomial distribution with a logit link function. Data were analyzed using the lmer package of R (version 4.2.2 (2022-10-31), R Core Team, Vienna, Austria). Results were considered significant with  $P \leq 0.05$  and were considered marginally significant with  $P \leq 0.10$ .

## **Results and Discussion**

From d 0 to 7 (phase 1; Table 3), the SBM × bacillus interaction was observed ( $P < 0.05$ ) for ADFI where pigs fed high SBM had decreased ADFI compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect ADFI. Additionally, a main effect of SBM level was observed, resulting in decreased ( $P < 0.05$ ) ADG and poorer F/G in pigs fed high SBM compared to pigs fed low SBM. For the d 7 BW, a main effect of SBM level was observed ( $P < 0.001$ ), resulting in decreased BW in pigs fed high SBM compared to pigs fed low SBM.

From d 7 to 21 (phase 2), the SBM  $\times$  bacillus interaction was observed ( $P < 0.05$ ) for ADG and F/G, where pigs fed high SBM had increased ADG and greater magnitude of improvement in F/G compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect ADG, and there was a smaller magnitude of improvement in F/G in pigs fed high SBM compared to those fed low SBM. Additionally, a main effect of SBM level was observed where pigs fed high SBM had decreased ( $P < 0.05$ ) ADFI compared to pigs fed low SBM. For the d 21 BW, the SBM  $\times$  bacillus interaction was observed ( $P = 0.015$ ), where pigs fed high SBM had increased BW compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect d 21 BW.

From d 21 to 42 (phase 3), there was a tendency for a main effect of bacillus, where pigs fed bacillus tended ( $P = 0.076$ ) to have increased ADG compared to those not fed bacillus. There were no treatment effects observed for ADFI or F/G. For the d 42 BW, the SBM  $\times$  bacillus interaction was observed ( $P = 0.026$ ) where the addition of bacillus increased BW in pigs fed low SBM; however, the addition of bacillus decreased BW in pigs fed high SBM.

From d 0 to 42 (overall), the SBM  $\times$  bacillus interaction was observed ( $P < 0.05$ ) for F/G, where pigs fed high SBM had improved F/G compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect F/G. A main effect of SBM level was observed where pigs fed high SBM had decreased ( $P < 0.05$ ) ADFI compared to pigs fed low SBM. No treatment effects were observed for ADG.

For fecal DM, a main effect of SBM level was observed where pigs fed high SBM had decreased ( $P < 0.05$ ) fecal DM compared to pigs fed low SBM.

For removals and mortality, the SBM  $\times$  bacillus interaction was observed ( $P < 0.05$ ) for removals, and a tendency for the SBM  $\times$  bacillus interaction was observed ( $P = 0.053$ ) for mortality, where pigs fed high SBM had numerically higher removals and mortality compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect removals or mortality. Additionally, the SBM  $\times$  bacillus interaction was observed ( $P < 0.05$ ) for total removals and mortality, where pigs fed high SBM had increased total removals and mortality compared to pigs fed low SBM when bacillus was not included in the diet; however, when bacillus was included in the diet, SBM level did not affect total removals and mortality.

In summary, high SBM levels had a negative effect on phase 1 growth performance, fecal DM, and removals and mortality, but in phase 2, high SBM levels improved ADG and phase 2 and 3 F/G. Additionally, when bacillus was not included in the diet, high SBM increased the removals and mortality; however, when bacillus was included in the diet, SBM level did not affect removals and mortality.

## Acknowledgments

Appreciation is expressed to Kent Nutrition Group (Muscatine, IA) and Hord Family Farms (Bucyrus, OH) who provided partial financial and technical support for this project.

*Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.*

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

<b>Item, %</b>	<b>Corn</b>	<b>Soybean meal</b>
DM	85.31	88.56
CP	7.53	46.50
Ether extract	2.88	2.35
Crude fiber	1.50	3.68
Ash	1.15	5.91
Essential AAs		
Arg	0.35	3.44
His	0.22	1.25
Ile	0.26	2.27
Leu	0.89	3.63
Lys	0.28	3.10
Met	0.16	0.66
Phe	0.38	2.44
Thr	0.27	1.83
Trp	0.05	0.65
Val	0.37	2.45
Non-essential AAs		
Ala	0.56	2.05
Asp	0.51	5.39
Cys	0.18	0.73
Glu	1.41	8.71
Gly	0.30	2.00
Pro	0.66	2.43
Ser	0.35	2.04
Tyr	0.20	1.66

<sup>1</sup>Ingredient samples were pooled and submitted for complete proximate and amino acid analysis in duplicate (University of Missouri Agricultural Experiment Station Chemical Laboratory; Columbia, MO). Analyzed values for corn and SBM were used in diet formulation.

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

Item	SBM level:	Phase 1		Phase 2		Phase 3	
		Low	High	Low	High	Low	High
Ingredient, %							
Corn		47.11	41.43	53.09	46.37	66.76	60.12
Soybean meal		24.16	30.43	28.96	36.45	28.62	36.01
Wheat		3.00	3.00	---	---	---	---
Wheat middlings		---	---	0.27	0.27	---	---
Whey powder		15.00	15.00	---	---	---	---
Whey permeate		---	---	12.75	12.75	---	---
Enzymatically treated soybean meal <sup>2</sup>		3.75	3.75	---	---	---	---
Corn oil		2.50	2.50	---	---	---	---
Calcium carbonate		0.65	0.67	0.88	0.91	1.04	1.06
Monocalcium phosphate		1.00	0.90	0.95	0.85	0.92	0.81
Sodium chloride		0.58	0.58	0.16	0.16	0.35	0.35
L-Lys-HCl		0.43	0.23	---	---	---	---
Liquid Lys 55%		---	---	0.67	0.32	0.68	0.33
DL-Met		0.25	0.19	0.25	0.18	0.22	0.15
L-Thr		0.24	0.15	---	---	---	---
ThrPro <sup>3</sup>		---	---	0.32	0.20	0.31	0.19
L-Trp		0.08	0.04	0.06	0.02	0.07	0.03
L-Val		0.12	---	0.13	---	0.10	---
Trace mineral premix		0.16	0.16	0.16	0.16	0.10	0.10
Vitamin premix		0.05	0.05	0.05	0.05	0.10	0.10
Zinc oxide		0.38	0.38	0.34	0.34	0.11	0.11
Copper sulfate		---	---	0.03	0.03	0.05	0.05
Phytase <sup>4</sup>		0.04	0.04	0.10	0.10	0.10	0.10
Choline chloride		0.04	0.04	---	---	---	---
Defusion <sup>5</sup>		0.50	0.50	---	---	---	---
Sodium metabisulfite		---	---	0.50	0.50	0.50	0.50
Denagard 10 <sup>6</sup>		---	---	0.18	0.18	---	---
Aureomycin 100 <sup>7</sup>		---	---	0.20	0.20	---	---
Bacillus-based feed additive <sup>8</sup>		-/+	-/+	-/+	-/+	-/+	-/+
Total		100	100	100	100	100	100

*continued*

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

Item	SBM level:	Phase 1		Phase 2		Phase 3	
		Low	High	Low	High	Low	High
Calculated analysis							
Standardized ileal digestible (SID) amino acids, %							
Lys		1.33	1.33	1.30	1.30	1.30	1.30
Ile:Lys		57	66	55	66	56	66
Leu:Lys		108	119	105	119	110	124
Met:Lys		38	35	38	35	37	34
Met and Cys:Lys		58	58	58	58	58	58
Thr:Lys		65	65	65	65	65	65
Trp:Lys		21	21	20	20	20	21
Val:Lys		71	71	70	72	70	73
His:Lys		33	37	33	38	34	40
Total Lys, %		1.49	1.50	1.44	1.46	1.45	1.47
NE, kcal/lb		1,096	1,078	1,067	1,047	1,098	1,079
SID Lys:NE, g/Mcal		5.20	5.28	5.34	5.44	5.37	5.47
CP, %		19.9	22.0	19.1	21.6	19.3	21.7
SID Lys:CP		6.69	6.06	6.79	6.02	6.74	5.98
Ca, %		0.65	0.66	0.69	0.71	0.68	0.70
STTD P, %		0.55	0.55	0.52	0.52	0.45	0.45
Ca:P		1.00	1.00	1.10	1.10	1.20	1.20
Analyzed composition <sup>9</sup>							
DM		90.2	90.3	88.9	89.0	86.9	87.3
CP		20.1	22.0	17.6	19.1	19.0	20.3
Crude fat		5.1	5.1	2.2	2.0	2.2	2.3
Crude fiber		1.9	2.4	2.1	2.4	2.4	2.7
Ash		5.9	6.4	6.6	7.1	5.3	5.9

<sup>1</sup>Phase 1 diets (pellet form) were fed from d 0 to 7, phase 2 diets (mash form) were fed from d 7 to 21, and phase 3 diets (mash form) were fed from d 21 to 42 of the study.

<sup>2</sup>HP300, Hamlet Protein, Denmark.

<sup>3</sup>CJ America Bio, Downers Grove, IL.

<sup>4</sup>Phase 1: Quantum Blue 5000 G (AB Vista, Plantation, FL), which provided an estimated release of 0.12% STTD P with 908 FTU/lb. Phase 2 and 3: Quantum Blue 2 G (AB Vista, Plantation, FL), which provided an estimated release of 0.12% STTD P with 908 FTU/lb.

<sup>5</sup>Promote, Lewisburg, OH.

<sup>6</sup>Elanco, Greenfield, IN.

<sup>7</sup>Phibro, Teaneck, NJ.

<sup>8</sup>Argis (Kent Nutrition Group, Muscatine, IA).

<sup>9</sup>Complete diet samples were collected from each feeder, then were pooled, ground to reduce particle size, and stored at -4°F until they were submitted for complete proximate analysis in duplicate (University of Missouri Agricultural Experiment Station Chemical Laboratory, Columbia, MO).

**Table 3. Effects of a bacillus-based feed additive and soybean meal (SBM) level on nursery pig growth performance<sup>1</sup>**

Item	Bacillus <sup>3</sup> :	Soybean meal <sup>2</sup>				SEM	<i>P</i> =		
		Low		High			SBM × bacillus	SBM	Bacillus
		-	+	-	+				
BW, lb									
d 0		14.7	14.7	14.7	14.7	0.21	0.882	0.976	0.929
d 7		16.0	16.0	15.9	15.8	0.21	0.847	< 0.001	0.350
d 21		29.9 <sup>b</sup>	30.2 <sup>ab</sup>	30.4 <sup>a</sup>	30.1 <sup>ab</sup>	0.41	0.015	0.080	0.919
d 42		63.2	64.2	63.9	63.7	0.59	0.026	0.638	0.201
d 0 to 7 (phase 1)									
ADG, lb		0.19	0.18	0.16	0.16	0.007	0.731	< 0.001	0.219
ADFI, lb		0.23 <sup>a</sup>	0.22 <sup>ab</sup>	0.21 <sup>b</sup>	0.21 <sup>ab</sup>	0.006	0.037	0.001	0.792
F/G		1.22	1.22	1.33	1.42	0.056	0.361	< 0.001	0.162
d 7 to 21 (phase 2)									
ADG, lb		0.98 <sup>b</sup>	1.00 <sup>ab</sup>	1.02 <sup>a</sup>	1.01 <sup>ab</sup>	0.018	0.035	0.001	0.904
ADFI, lb		1.18	1.18	1.15	1.16	0.021	0.949	0.003	0.616
F/G		1.20 <sup>a</sup>	1.18 <sup>a</sup>	1.13 <sup>c</sup>	1.15 <sup>b</sup>	0.009	0.003	< 0.001	0.506
d 21 to 42 (phase 3)									
ADG, lb		1.58	1.62	1.59	1.59	0.012	0.110	0.436	0.076
ADFI, lb		2.35	2.40	2.36	2.36	0.024	0.119	0.372	0.113
F/G		1.49	1.48	1.48	1.48	0.008	0.937	0.789	0.682
d 0 to 42 (overall)									
ADG, lb		1.15	1.16	1.16	1.15	0.010	0.107	0.861	0.278
ADFI, lb		1.60	1.62	1.59	1.59	0.017	0.500	0.035	0.208
F/G		1.39 <sup>a</sup>	1.39 <sup>ab</sup>	1.37 <sup>c</sup>	1.38 <sup>bc</sup>	0.005	0.014	< 0.001	0.505
Fecal DM <sup>4</sup> , %		20.3	20.1	18.9	18.2	0.61	0.672	0.008	0.486
Removals, %		1.9	3.4	3.7	3.1	0.50	0.034	0.014	0.363
Mortality, %		0.1	0.4	0.7	0.3	0.20	0.053	0.017	0.087
Total removals and mortality, %		2.0 <sup>b</sup>	3.8 <sup>ab</sup>	4.4 <sup>a</sup>	3.3 <sup>ab</sup>	0.60	0.011	0.003	0.166

<sup>a,b,c</sup>Means with different superscripts differ, *P* < 0.05.

<sup>1</sup>A total of 4,320 nursery pigs (PIC 337 × 1050; initially 14.7 ± 0.21 lb) were used in a 42-d growth study with 54 pigs per feeder (double-sided) and 20 feeders per treatment.

<sup>2</sup>Low SBM diets contained 24.16, 28.96, and 28.62% SBM in phases 1, 2, and 3, respectively. High SBM diets contained 30.43, 36.45, and 36.01% SBM in phases 1, 2, and 3, respectively.

<sup>3</sup>Argis; Kent Nutrition Group, Muscatine, IA. The bacillus-based feed additive was added to the diet to reach 10<sup>6</sup> CFU/g.

<sup>4</sup>Fecal samples were collected from three pigs per feeder (60 pigs per treatment) on approximately d 14 (range of 9 to 20 d post-placement) of the study to determine fecal DM.