Kansas Agricultural Experiment Station Research Reports

Volume 4
Issue 9 *Swine Day*

Article 4

2018

Effects of a Bacillus-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora

M. B. Menegat
Department of Diagnostic Medicine/Pathobiology, Kansas State University, mbmenegat@ksu.edu

K. M. Gourley
Kansas State University, Manhattan, kgourley@k-state.edu

M. B. Braun
Kansas State University, mbraun1@ksu.edu

See next page for additional authors

Recommended Citation

Menegat, M. B.; Gourley, K. M.; Braun, M. B.; DeRouchey, J. M.; Woodworth, J. C.; Bryte, J.; Tokach, M. D.; Dritz, S. S.; and Goodband, R. D. (2018) "Effects of a Bacillus-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora," *Kansas Agricultural Experiment Station Research Reports*: Vol. 4: Iss. 9. https://doi.org/10.4148/2378-5977.7652

Effects of a Bacillus-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora

Funding Source

Appreciation is expressed to Quality Technology International, Inc. (Elgin, IL) and Calpis Co., Ltd. (Tokyo, Japan) for financial support and microbial analysis.

Authors

M. B. Menegat, K. M. Gourley, M. B. Braun, J. M. DeRouchey, J. C. Woodworth, J. Bryte, M. D. Tokach, S. S. Dritz, and R. D. Goodband

K-STATE Research and Extension

Swine Day 2018

Kansas State University Swine Industry Day 2018

Effects of a *Bacillus*-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora[1]

M.B. Menegat,[2] K.M. Gourley, M.B. Braun, J.M. DeRouchey, J.C. Woodworth, J. Bryte,[3] M.D. Tokach, S.S. Dritz,[2] and R.D. Goodband

Summary

The objective of this study was to evaluate the effects of supplementation of *Bacillus subtilis* C-3102 on sow performance and fecal microflora and on progeny growth performance, fecal consistency, and fecal microflora. For the sow portion of this study, a total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used from d 30 of gestation until weaning (d 19 of lactation). Treatments consisted of providing a control diet (n = 14 sows) or a probiotic diet (n = 15 sows) supplemented with *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) at 500,000 CFU/g of complete feed in gestation and 1,000,000 CFU/g of complete feed in lactation. For the nursery portion of the study, a total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE) progeny of the sows on study, were used in a 42-d nursery trial. There were 4 or 5 pigs per pen and 18 or 19 replications per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control diet vs. probiotic diet) and nursery treatment (control diet vs. probiotic diet). In the nursery probiotic diet, a combination of the probiotic *Bacillus subtilis* C-3102 and prebiotics based on beta glucans and mannan oligosaccharides (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) was included at 0.05% of complete feed. Fecal scoring was used to categorize fecal consistency of nursing litters and nursery pens. Fecal samples were collected from sows and piglets for microbial analysis performed by culture method and bacterial quantification. The results demonstrate that sows fed the probiotic diet had a marginally significant ($P = 0.056$) increase in lactation average daily feed intake (ADFI), consuming on average 0.6 lb more feed per day than sows fed the control diet, but it did not result ($P > 0.10$) in improvement in sow or piglet body weight (BW) at weaning. Sows fed the probiotic diet had marginally significant ($P = 0.060$) larger litter size after equalization on d 2 after birth, with on average 0.5 more piglet per litter than sows fed the control diet, but it did not result ($P > 0.10$) in

[1]Appreciation is expressed to Quality Technology International, Inc. (Elgin, IL) and Calpis Co., Ltd. (Tokyo, Japan) for financial support and microbial analysis.

[2]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

[3]Quality Technology International, Inc. (Elgin, IL).

larger litter size at weaning. In the nursery, there was no evidence for effect of sow treatment, nursery treatment, or interactions ($P > 0.10$) on overall growth performance. However, growth performance from d 21 to 42 and final nursery BW were greater ($P < 0.05$) in pigs from sows fed the control diet compared to the probiotic diet. The evaluation of fecal score in nursing and nursery pigs indicated that fecal consistency was not influenced ($P > 0.10$) by sow or pig diet. Microbial analysis revealed an increase ($P < 0.01$) in number of *Bacillus subtilis* C-3102 and, consequently, total *Bacillus* sp. in fecal microflora of sows and nursery pigs fed the probiotic diet. Also, piglets that were born and nursed by sows fed a probiotic diet also displayed this change ($P < 0.01$) in fecal microbial population before weaning. In conclusion, the findings of this study demonstrate a potential benefit of providing *Bacillus subtilis* C-3102 to sows during gestation and lactation on lactation feed intake. However, the probiotic inclusion to sow diets impaired growth performance and BW of the progeny in late nursery. The probiotic diet provided to sows or nursery pigs did not influence fecal consistency or number of potentially harmful bacteria in fecal microflora of sows and pigs. However, the probiotic diet was able to induce a change in fecal microbial population in sows, nursing piglets, and nursery pigs by increasing the number of total *Bacillus* sp. The effects of *Bacillus subtilis* C-3102 on litter size after equalization require further elucidation in studies with larger number of sows and litters.

Introduction

Probiotics are non-pathogenic live microorganisms that if provided in adequate amounts can improve the intestinal microbial balance and benefit the host.[4] Probiotics have been explored as a dietary feed additive to improve performance and preserve intestinal health while minimizing the use of antibiotics. The use of probiotics has also been explored in sow diets as a means of modulating the developing intestinal microbiota of neonatal pigs.[5] Furthermore, it has been suggested that the beneficial effects of probiotics might be enhanced during stressful periods, such as farrowing, lactation, and weaning.[6]

Bacillus sp. are Gram-positive spore-forming bacteria typically used as probiotics for swine in single strain and multi-strain preparations. Spores are considered stable during feed manufacturing and storage, and after ingestion can germinate but not proliferate in the intestine. In sows, supplementation of *Bacillus subtilis* C-3102 has been associated with improvement of reproductive performance,[7] reduction of occurrence of diarrhea in newborn pigs,[8] and reduction of pathogens in sow fecal microflora by increasing the populations of beneficial bacteria, particularly the *Lactobacillus* sp.[6] In pigs, diets with

[4]Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.
[5]Baker, A.A., Davis, E., Spencer, J. D., Moser, R., Rehberger, T. 2013. The effect of a *Bacillus*-based direct-fed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. Anim. Reprod. Sci. 91:3390–3399.
[6]Chaucheyras-Durand, F., Durand, H. 2010. Probiotics in animal nutrition and health. Benef. Microbes. 1:3–9.
[7]Kritas, S.K., Marubashi, T., Filioussis, G., Petridou, E., Christodoulopoulos, G., Burriel, A.R., Tzivara, A., Theodoridis, A., and Pískoriková, M. 2015. Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (*Bacillus subtilis* C-3102). J. Anim. Sci. 93:405-413.
[8]Maruta, K., Miyazaki, H., Tadano, Y., Masuda, S., Suzuki, A, Takahashi, H., and Takahashi, M. 1996. Effects of *Bacillus subtilis* C-3102 intake on fecal flora of sows and on diarrhea and mortality rate of their piglets. Anim. Sci. Technol. 67(5):403-409.

Bacillus subtilis C-3102 have been shown to reduce pathogens in fecal microflora of nursing pigs,[6] increase weaning weight,[6] and improve nursery growth performance.[9]

The potential benefits of providing *Bacillus subtilis* C-3102 in swine diets have prompted the interest of investigating its effect on sows and their progeny through the nursery period. Therefore, the objective of this study was to evaluate the effects of supplementation of *Bacillus subtilis* C-3102 on sow performance and fecal microflora and on progeny growth performance, fecal consistency, and fecal microflora.

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 University Swine Teaching and Research Center in Manhattan, KS. A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and progeny were used in the study. This study was divided in a sow portion, from d 30 of gestation to sow weaning, and a nursery portion, from weaning to d 42 of nursery.

Sow Portion

For the sow portion of this study, sows were individually housed in environmentally-controlled and mechanically-ventilated barns during gestation and lactation. A total of 29 sows with confirmed pregnancy on d 30 of gestation were assigned to dietary treatments in a randomized complete block design based on parity and BW at the beginning of experiment. Dietary treatments consisted of a control diet (n = 14 sows) or a probiotic diet (n = 15 sows) supplemented with *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan).

Gestation diets were fed from d 30 of gestation until farrowing. Treatments were top dressed in a common gestation diet according to daily feed allowance. Sows were fed 4.5, 5.5, or 6.5 lb/d of gestation diet according to body condition from d 30 to 112 of gestation. On d 112 of gestation, sows were moved to the farrowing house and fed 6.0 lb/d of gestation diet until farrowing. In the control diet, the top dress contained ground corn. In the probiotic diet, the top dress contained ground corn and Calsporin® to achieve 500,000 CFU/g of complete feed in gestation.

Lactation diets were fed from farrowing to weaning at approximately d 19 of lactation. Treatments were incorporated into the diet formulation in lactation. Sows were allowed *ad libitum* feed intake during lactation with daily feed delivery and recording by an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, Quebec City, Canada). In the probiotic diet, Calsporin® was included to achieve 1,000,000 CFU/g of complete feed in lactation.

During lactation, cross-fostering of piglets was performed to equalize litter size within sow treatment group within 24 h after birth. Nursing piglets were provided with a heat lamp and access to water, but no creep feeding.

[9]Marubashi, T., Gracia, M.I., Vilà, B., Bontempo, V., Kritas, S.K., and Piskoríková, M. 2012. The efficacy of the probiotic feed additive Calsporin® (*Bacillus subtilis* C-3102) in weaned piglets: Combined analysis of four different studies. J. Appl. Anim. Nut. 1:1-5.

Sow performance was determined by recording feed intake on a daily basis and BW on d 30 and 112 of gestation and d 19 of lactation. Additionally, fecal samples were collected from sows for microbial analysis on d 30 and 112 of gestation and d 18 of lactation. Farrowing and litter performance were assessed by recording number of piglets total born, born alive, and stillborn; individual piglet BW at birth, d 2, 12, and 19; litter size at d 2, 12, and 19; and survivability until weaning. Additionally, on d 2 and 18, fecal scoring was conducted to characterize consistency of piglets feces, and fecal samples were collected from piglets for microbial analysis.

Fecal scoring categorized the consistency of piglets' feces per litter using a numerical scale from 1 to 5, as follows: 1) hard feces; 2) firm formed feces; 3) soft moist feces that retain shape; 4) soft unformed feces; and 5) watery feces. Fecal scoring was performed by 3 trained individuals and the concordant score was considered as the litter score.

Fecal samples were collected directly from the rectum of sows and piglets for microbial analysis. In the case of piglets, fecal samples were pooled and analyzed by litter. Microbial analysis of fecal samples was performed by culture method and quantification ($\log_{10}$ CFU/g) of *Bacillus subtilis* C-3102 (Calsporin®), total *Bacillus* sp., *Lactobacillus* sp., *Clostridium perfringens*, *Salmonella* spp., *Enterococcus* sp., Enterobacteriaceae, total aerobes, and total anaerobes. Limit of detection was 2×10^2. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc. (Peachtree City, GA).

Diets were based on corn and soybean meal and were fed in meal form (Table 1). Diets were formulated to meet or exceed the National Research Council (NRC)[10] nutrient requirements, and Calsporin® was included in the diet at the expense of corn. Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Diet samples were collected at manufacturing, and composite samples were submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and quantification of *Bacillus subtilis* C-3102 (Calsporin®; Calpis America, Inc., Peachtree City, GA).

Data were analyzed using a linear mixed model. Treatment was included as fixed effect and block as random effect. Sow or litter were the experimental units. Born alive and stillborn as a proportion of total piglets born, and pre-wean mortality were analyzed assuming a binomial distribution. Fecal score was analyzed assuming a multinomial distribution. Fecal score and microbial analysis were analyzed as repeated measures. Statistical models were fitted using the GLIMMIX procedure of SAS® version 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Nursery portion

A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Only nine weaned pigs (5 from control litters and 4 from probiotic litters) were not included in the nursery study due to poor health condition. The experimental period comprised a

[10]National Research Council. 2012. Nutrient Requirements of Swine. 11th Rev. Ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

42-d period into the nursery starting at weaning. Pigs were allotted to pens and pens to treatments in a completely randomized design based on BW at weaning. There were 4 or 5 pigs per pen and 18 or 19 replications per treatment. Nursery pigs were housed in 4 × 4 ft pens with a 4-hole dry self-feeder and one cup waterer.

Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control diet vs. probiotic diet) and nursery treatment (control diet vs. probiotic diet). In the nursery probiotic diet, a combination of the probiotic *Bacillus subtilis* C-3102 and prebiotics based on beta glucans and mannan oligosaccharides (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) was included at 0.05% of complete feed in the nursery, which corresponds to 500,000 CFU of *Bacillus subtilis* C-3102 per gram of complete feed.

Nursery performance was assessed by recording BW, feed disappearance, and fecal score on d 0, 7, 14, 21, 28, 35, and 42 to determine average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (F/G), and fecal consistency. Additionally, on d 21 and 42, fecal samples were collected from piglets for microbial analysis.

Fecal scoring categorized the consistency of pigs' feces per pen using a numerical scale from 1 to 5, as follows: 1) hard feces; 2) firm formed feces; 3) soft moist feces that retain shape; 4) soft unformed feces; and 5) watery feces. Fecal scoring was performed by 3 trained individuals and the concordant score was considered as the pen score.

Fecal samples were collected directly from the rectum of pigs for microbial analysis. Fecal samples were collected from two pigs per pen and three pens of the same treatment were pooled for analysis (n = 24). Microbial analysis of fecal samples was performed by culture method and quantification ($\log_{10}$ CFU/g) of *Bacillus subtilis* C-3102 (Calsporin®), total *Bacillus* sp., *Lactobacillus* sp., *Clostridium perfringens*, *Salmonella* spp., *Enterococcus* sp., Enterobacteriaceae, total aerobes, and total anaerobes. Limit of detection was 2×10^2. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc. (Peachtree City, GA).

Diets were based on corn and soybean meal and were fed in three dietary phases: Phase 1, fed from d 0 to 7 in pellet form; Phase 2, fed from d 7 to 21 in meal form; and Phase 3, fed from d 21 to 42 in meal form (Table 2). Diets were formulated to meet or exceed the NRC[10] nutrient requirements, and BacPack ABF™ was included in the diet at the expense of corn. Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Diet samples were collected at manufacturing, and composite samples were submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and quantification of *Bacillus subtilis* C-3102 (Calsporin®; Calpis America, Inc., Peachtree City, GA).

Data were analyzed using a linear mixed model. Treatment was included as fixed effect and pen as the experimental unit. Preplanned contrast statements were built to evaluate the main effects and interactions of sow treatment and nursery treatment. Fecal score was analyzed assuming a multinomial distribution. Fecal score and microbial analysis were analyzed as repeated measures. Statistical models were fitted using the GLIMMIX

procedure of SAS® version 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

The analyzed dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), fat, Ca, P, and *Bacillus subtilis* C-3102 content of experimental diets (Table 3) were consistent with formulated estimates. The presence of *Bacillus subtilis* C-3102 in control diets is associated to the ubiquitous nature of this species. The levels in control diets were within expectations and in accordance to the literature,[11] i.e. at least 1 $\log_{10}$ lower CFU/g compared to probiotic diets.

Sow portion

Dietary addition of *Bacillus subtilis* C-3102 to sows during gestation and lactation did not influence ($P > 0.10$) sow BW at the end of gestation or at weaning (Table 4). There was no evidence for difference ($P > 0.10$) on number of piglets total born, born alive, stillborn, or piglet birth weight between sows fed control or probiotic diets. Sows fed the probiotic diet had a marginally significant ($P = 0.056$) increase in ADFI during lactation, consuming on average 0.6 lb more feed per day than sows fed the control diet. Interestingly, the increase in feed intake on probiotic-fed sows did not result ($P > 0.10$) in improvement in piglet BW at weaning, piglet ADG during lactation, pre-weaning mortality, or sow BW change from farrowing to weaning.

Sows fed the probiotic diet had marginally significant ($P = 0.060$) larger litter size on d 2 after birth, with on average 0.5 more piglet per litter than sows fed the control diet. This improvement in litter size resulted from the numeric increase ($P = 0.624$) on number of piglets born alive in probiotic-fed sows, with on average 0.4 more piglet born alive than sows fed the control diet. Probably, the variation in litter size prevented finding evidence for differences at birth, whereas the consistency in litter size after equalization allowed for a significant response. However, the probiotic treatment did not result ($P > 0.10$) in larger litter size at weaning.

Fecal score of nursing piglets was not influenced ($P > 0.10$) by dietary addition of *Bacillus subtilis* C-3102 to sows during gestation and lactation (Figure 1). Fecal consistency was mostly classified as hard feces or firm formed feces in litters from both probiotic- or control-fed sows. On d 2, fecal consistency was mostly classified as firm formed feces or soft moist feces, but on d 18 fecal consistency was mostly shifted to hard feces or firm formed feces ($P = 0.070$).

Analysis of nursing piglet fecal microflora revealed a change ($P < 0.05$) in number of *Bacillus subtilis* C-3102, total *Bacillus* sp., and *Lactobacillus* sp. in litters from sows fed the probiotic (Table 5). The fecal microflora of 2-day-old piglets contained similar level of *Bacillus subtilis* C-3102 regardless of sow diet, but only piglets from sows receiving the probiotic diet increased ($P < 0.001$) the number of *Bacillus subtilis* C-3102 on d 18 of lactation. Similarly, the fecal microflora of 2-day-old piglets contained similar level of total *Bacillus* sp., but piglets from sows receiving the probiotic diet increased

[11]Marubashi, T., Gracia, M.I., Vilà, B., Bontempo, V., Kritas, S.K., and Piskoríková, M. 2012. The efficacy of the probiotic feed additive Calsporin® (*Bacillus subtilis* C-3102) in weaned piglets: Combined analysis of four different studies. J. Appl. Anim. Nut. 1:1-5.

($P = 0.007$) the number of total *Bacillus* sp. on d 18 of lactation, while piglets from sows receiving the control diet reduced the number of total *Bacillus* sp. in the same period. The number of *Lactobacillus* sp. remained constant during lactation in piglets from probiotic-fed sows while the number increased from d 2 to 18 of lactation in piglets from control-fed sows. However, there was a similar level of *Lactobacillus* sp. in fecal microflora of piglets regardless of sow diet on d 18.

As duration of lactation increased, microbial analysis revealed a decrease ($P < 0.10$) from d 2 to 18 of lactation in levels of *Clostridium perfringens* (8.93 to 8.57 $\log_{10}$ CFU/g), Enterobacteriaceae (9.30 to 8.38 $\log_{10}$ CFU/g), total aerobes (8.23 to 6.70 $\log_{10}$ CFU/g), and total anaerobes (9.43 to 8.60 $\log_{10}$ CFU/g) in litters of both control- and probiotic-fed sows. The number of *Enterococcus* sp. in fecal microflora of piglets was not affected ($P > 0.10$) by sow diet or day of lactation. *Salmonella* spp. was detected on d 2 of lactation in one out of 13 fecal samples from litters from control-fed sows (7.33 $\log_{10}$ CFU/g), but it was not detectable on d 18 of lactation.

Analysis of sow fecal microflora revealed a change ($P < 0.01$) on number of *Bacillus subtilis* C-3102 and total *Bacillus* sp. in sows fed Calsporin® (Table 6). In sows receiving the probiotic diet, the level of *Bacillus subtilis* C-3102 and total *Bacillus* sp. increased ($P < 0.01$) during gestation, from d 30 until d 113 of gestation, and then remained at a constant level in lactation until weaning. Whereas in sows receiving the control diet, the level of *Bacillus subtilis* C-3102 and total *Bacillus* sp. either decreased or remained at a constant level during gestation and lactation. Both the number of *Bacillus subtilis* C-3102 and total *Bacillus* sp. were increased ($P < 0.01$) in probiotic-fed sows compared to control sows at any stage of gestation and lactation.

There was a change ($P < 0.001$) on sow fecal microflora during the course of gestation and lactation in the levels of *Lactobacillus* sp., *Clostridium perfringens*, Enterobacteriaceae, and total anaerobes regardless of sow diet. The number of *Lactobacillus* sp. remained constant during gestation (7.13 and 6.84 $\log_{10}$ CFU/g on d 30 and 113), but increased during lactation (8.45 $\log_{10}$ CFU/g on d 18; $P < 0.001$). The number of *Clostridium perfringens* in fecal microflora decreased during the course of gestation and lactation (8.03, 7.74, and 6.08 $\log_{10}$ CFU/g on d 30 of gestation, d 113 of gestation, and d 18 of lactation, respectively; $P < 0.001$). Enterobacteriaceae remained at a constant level during gestation (7.48 and 7.36 $\log_{10}$ CFU/g on d 30 and 113), but decreased during lactation (6.57 $\log_{10}$ CFU/g on d 18; $P < 0.001$). The number of total anaerobes reduced during gestation (9.15 and 9.00 $\log_{10}$ CFU/g on d 30 and 113), but returned to increased levels during lactation (9.30 $\log_{10}$ CFU/g on d 18; $P = 0.001$).

Salmonella spp. was detected on d 113 of gestation in two out of 14 fecal samples from control-fed sows (average 5.49 $\log_{10}$ CFU/g) and in one out of 15 fecal samples from probiotic-fed sows (4.34 $\log_{10}$ CFU/g), but it was not detectable on d 30 of gestation and d 18 of lactation. *Enterococcus* sp. was not analyzed in sow fecal samples.

The findings of the sow portion of the study demonstrate a potential benefit of providing *Bacillus subtilis* C-3102 to sows during gestation and lactation on lactation feed intake. Studies with larger number of sows and litters would contribute to further elucidate the effects of this probiotic on litter size after equalization. Moreover,

providing the probiotic to sows during gestation and lactation induced a change in fecal microbial population by increasing the number of *Bacillus subtilis* C-3102 and, consequently, total *Bacillus* sp. Interestingly, the sow fecal microflora was found to have an important influence on piglet fecal microflora during lactation. Piglets that were born and nursed by sows fed a probiotic diet also displayed a shift in fecal microbial population with greater counts of *Bacillus subtilis* C-3102 and total *Bacillus* sp. before weaning. Although the change on fecal microflora did not impact piglet growth performance, fecal consistency, or number of potentially harmful bacteria in this study, it demonstrates the promise of using the sow diet as a means of modulating microbial population in the piglet.

Nursery portion

There was no evidence ($P > 0.10$) for interactive effects of sow treatment and nursery treatment on growth performance of nursery pigs (Table 7). Therefore, the main effects of sow treatment and nursery treatment on growth performance of nursery pigs were further explored (Table 8).

In Phase 1 (d 0 to 7), there was no evidence ($P > 0.10$) for effect of sow treatment on pig growth performance. There was a marginally significant ($P = 0.084$) effect of nursery treatment on ADG, with pigs fed the probiotic diet in the nursery having increased ADG in Phase 1 compared to pigs fed the control diet. However, no evidence ($P > 0.10$) for effect of nursery treatment was observed on ADFI or F/G. In Phase 2 (d 7 to 21), there was no evidence ($P > 0.10$) for effect of sow treatment or nursery treatment on growth performance.

In Phase 3, (d 21 to 42), there was an effect ($P < 0.01$) of sow treatment on ADG and ADFI, with pigs born from sows fed the control diet having increased ADG and ADFI compared to pigs born from sows fed the probiotic diet. Moreover, there was a marginally significant ($P = 0.088$) effect of nursery treatment on F/G, with improvement in F/G observed in pigs fed the control diet over the probiotic diet. However, no evidence ($P > 0.10$) for effect of nursery treatment was observed on ADG or ADFI.

Overall (d 0 to 42 post-weaning), there was no evidence ($P > 0.10$) for effect of sow or nursery treatment on pig growth performance. Also, there was no evidence ($P > 0.10$) for effect of nursery treatment on final BW. However, there was an effect ($P = 0.042$) of sow treatment, where BW at the end of nursery was greater in pigs from sows fed the control diet rather than the probiotic diet.

Fecal score of nursery pigs was not influenced ($P > 0.10$) by sow dietary treatment, nursery dietary treatment, or their interaction (Figure 2). Fecal consistency was mostly classified as soft moist feces or soft unformed feces across the treatments. During the 42-d nursery period, fecal consistency gradually shifted to a looser pattern (Figure 3; $P = 0.001$). From d 28 on, there is an increase in frequency distribution of pens with soft unformed feces, absence of pens with firm formed feces, and notice of pens with watery feces on d 42.

Microbial analysis revealed a change ($P = 0.009$) in level of *Bacillus subtilis* C-3102 on nursery pig fecal microflora by the interaction of sow treatment, nursery treatment, and

day in nursery (Table 9). Before weaning, piglets from sows fed the probiotic diet had higher ($P < 0.001$) levels of *Bacillus subtilis* C-3102 in fecal microflora than piglets from sows fed the control diet (Table 5). In the nursery, pigs from control-fed sows that were fed a control diet in the nursery maintained lower levels of *Bacillus subtilis* C-3102; whereas pigs from control-fed sows that were fed a probiotic diet in the nursery rapidly increased the levels of *Bacillus subtilis* C-3102 in fecal microflora. Similarly, pigs from probiotic-fed sows that were fed a probiotic diet in the nursery maintained higher levels of *Bacillus subtilis* C-3102 during nursery; whereas pigs from probiotic-fed sows that were fed a control diet in the nursery gradually decreased the levels of *Bacillus subtilis* C-3102 in fecal microflora.

Before weaning, piglets from sows fed the probiotic diet had higher ($P = 0.007$) level of total *Bacillus* sp. in fecal microflora than piglets from sows fed the control diet (Table 5). However, no evidence ($P > 0.10$) for effect of sow treatment on number of total *Bacillus* sp. was observed after weaning. Still, the number of total *Bacillus* sp. was greater ($P < 0.001$) in pigs fed the probiotic diet compared to the control diet in the nursery (5.69 vs. 4.09 $\log_{10}$ CFU/g, respectively).

Before weaning, there was no evidence ($P > 0.10$) for effect of sow treatment on number of total aerobes on nursing pig fecal microflora (Table 5). However, after weaning, pigs from control-fed sows had higher ($P = 0.022$) number of total aerobes in fecal microflora than pigs from probiotic-fed sows (9.65 vs. 9.54 $\log_{10}$ CFU/g, respectively). Moreover, pigs fed the control diet had increased ($P = 0.036$) total number of aerobes during nursery (9.52 to 9.70 $\log_{10}$ CFU/g from d 21 to 42); whereas pigs fed the probiotic diet maintained a constant number of total aerobes during nursery (9.58 and 9.57 CFU/g on d 21 and 42, respectively).

There was an interaction ($P = 0.012$) between sow treatment and nursery treatment on number of total anaerobes in nursery pig fecal microflora. Pigs born from sows fed the control diet that were also fed the control diet in the nursery had higher number of total anaerobes (10.23 $\log_{10}$ CFU/g) compared to pigs that were either fed the probiotic diet in the nursery (10.11 $\log_{10}$ CFU/g) or born from sows fed the probiotic diet (10.10 $\log_{10}$ CFU/g). Number of total anaerobes in pigs born from sows fed the probiotic diet that were also fed the probiotic diet in the nursery was intermediate (10.17 $\log_{10}$ CFU/g). Moreover, number of total anaerobes decreased ($P = 0.038$) from d 21 to 42 of nursery (10.19 to 10.12 $\log_{10}$ CFU/g) regardless of dietary treatment.

The levels of *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae in fecal microflora were only marginally significantly affected by main effects or interactions of sow treatment, nursery treatment, and day in the nursery (Table 10). The practical and biological significance of these changes are not considered relevant to the study. *Clostridium perfringens* and *Salmonella* spp. were not detectable on d 21 and 42 of nursery.

The findings of the nursery portion of the study indicate a similar overall growth performance and fecal consistency in nursery pigs in spite of probiotic inclusion in sow diet and/or nursery. However, providing *Bacillus subtilis* C-3102 to sows during gestation and lactation reduced growth performance and BW of the progeny in late nursery. Although the reason for impairment of growth performance remains unclear,

it does not seem to be related to alterations of fecal consistency or fecal microflora. The probiotic diet fed to sows did not influence fecal consistency in the nursery and was only found to increase the population of total aerobes in fecal microflora of nursery pigs, which is consistent with an increase in number of *Bacillus subtilis* C-3102. The potentially harmful bacteria in fecal microflora remained at a similar level in pigs fed the control or probiotic diet. The inclusion of pharmacological levels of zinc oxide in nursery diets, as well as health status and sanitation of nursery facilities might have contributed to the characteristics of fecal microflora found in this trial.

The number of *Bacillus subtilis* C-3102 on fecal microflora seemed to be dependent on continuous supplementation of a probiotic source in the diet, i.e. Calsporin® or BacPack ABF™. Although colonization of *Bacillus subtilis* C-3102 was detected at weaning in pigs from sows fed the probiotic diet, the population of *Bacillus subtilis* C-3102 gradually decreased in the nursery when the probiotic diet was not provided. At the same time, the number of *Bacillus subtilis* C-3102 rapidly increased when a probiotic diet was provided to pigs with a small population of *Bacillus subtilis* C-3102.

Conclusion

In conclusion, the findings of this study demonstrate a potential benefit of providing *Bacillus subtilis* C-3102 to sows during gestation and lactation on lactation feed intake, but more commercial validation is needed. However, the probiotic inclusion to sow diets impaired growth performance and BW of the progeny in late nursery. The probiotic diet provided to sows or nursery pigs did not influence fecal consistency or number of potentially harmful bacteria in fecal microflora of sows and pigs. However, the probiotic diet was able to induce a change in fecal microbial population in sows, nursing piglets, and nursery pigs by increasing the number of total *Bacillus* sp. The effects of *Bacillus subtilis* C-3102 on litter size after equalization require further elucidation in studies with larger number of sows and litters.

Table 1. Composition of gestation and lactation diets (as-fed basis)[1]

Item	Gestation[2]	Lactation[3]
Ingredient, %		
Corn	80.40	63.43
Soybean meal, 47% crude protein	15.61	30.56
Choice white grease	---	2.50
Calcium carbonate	1.15	0.90
Monocalcium phosphate, 21.5% aP	1.40	1.05
Sodium chloride	0.50	0.50
L-Lysine HCl	---	0.20
DL-Methionine	---	0.05
L-Threonine	0.03	0.10
L-Valine	---	0.05
Vitamin premix	0.25	0.25
Trace mineral premix	0.15	0.15
Sow add pack	0.50	0.25
Phytase[4]	0.02	0.02
Calsporin 1.0B[5]	---	+/-
Total	100.0	100.0

continued

Table 1. Composition of gestation and lactation diets (as-fed basis)[1]

Item	Gestation[2]	Lactation[3]
Calculated analysis		
Standardized ileal digestible (SID) amino acids, %		
Lysine	0.56	1.08
Isoleucine:lysine	86	67
Leucine: lysine	209	139
Methionine:lysine	38	30
Methionine and cysteine:lysine	76	56
Threonine:lysine	79	67
Tryptophan:lysine	24	20
Valine:lysine	99	78
Total lysine, %	0.66	1.22
ME, kcal/lb	1,472	1,534
NE, kcal/lb	1,123	1,145
SID lysine:NE, g/Mcal	2.26	4.28
Crude protein, %	14.1	20.1
Calcium, %	0.85	0.75
STTD P, %	0.48	0.44

[1]Gestation diet was fed from d 30 of gestation until farrowing and lactation diets were fed from farrowing until weaning on d 19 of lactation. Diets were fed in meal form.
[2]Treatments were top dressed in a common gestation diet. In the control diet, the top dress contained ground corn. In the probiotic diet, the top dress contained ground corn and Calsporin® to achieve 500,000 CFU/g of complete feed in gestation.
[3]In the probiotic diet, Calsporin® was included to achieve 1,000,000 CFU/g of complete feed in lactation at the expense of corn.
[4]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 FTU/lb and an estimated release of 0.10% available P.
[5]Calsporin 1.0B (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial product based on viable spores of *Bacillus subtilis* C-3102 at concentration 1×10^9 CFU/g of product.
ME = metabolizable energy.
NE = net energy.
STTD = standardized total tract digestible.
+/- Inclusion rate of Calsporin 1.0B in the probiotic diet in lactation was 0.10%.

Table 2. Composition of nursery diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn	42.97	55.17	60.84
Soybean meal, 47% crude protein	18.78	24.81	34.61
Whey powder	25.00	10.00	---
Fish meal	4.50	---	---
HP 300[2]	2.50	5.00	---
Choice white grease	3.00	1.00	1.00
Calcium carbonate	0.40	0.73	0.85
Monocalcium phosphate, 21.5% aP	0.60	1.10	1.00
Sodium chloride	0.30	0.55	0.60
L-Lysine-HCl	0.45	0.45	0.35
DL-Methionine	0.22	0.22	0.15
L-Threonine	0.20	0.19	0.14
L-Tryptophan	0.05	0.03	0.01
L-Valine	0.15	0.10	0.04
Vitamin premix	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15
Vitamin E, 20,000 IU	0.05	---	---
Choline chloride 60%	0.04	---	---
Phytase[3]	0.02	0.02	0.02
Zinc oxide	0.39	0.25	---
BacPack ABF[4]	+/-	+/-	+/-
Total	100.0	100.0	100.0

continued

Table 2. Composition of nursery diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lysine	1.40	1.35	1.30
Isoleucine:lysine	55	58	61
Leucine: lysine	107	115	124
Methionine:lysine	37	37	34
Methionine and cystine:lysine	56	58	57
Threonine:lysine	63	63	63
Tryptophan:lysine	19.3	19.1	19.0
Valine:lysine	69	69	69
Histidine:lysine	31	36	40
Total lysine, %	1.53	1.49	1.45
ME, kcal/lb	1,575	1,514	1,505
NE, kcal/lb	1,194	1,127	1,108
SID lysine:NE, g/Mcal	5.30	5.44	5.32
Crude protein, %	20.5	21.1	22.1
Calcium, %	0.75	0.70	0.70
STTD P, %	0.49	0.43	0.36

[1]Nursery diets were fed in three dietary phases: Phase 1, from d 0 to 7 in pellet form; Phase 2, from d 7 to 21 in meal form; and Phase 3, from d 21 to 42 in meal form.
[2]Hamlet Protein, Inc., Findlay, OH.
[3]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.
[4]BacPack ABF™ (Quality Technology International, Inc., Elgin, IL) is a product containing the probiotic *Bacillus subtilis* C-3102 and prebiotics based on beta glucans and mannan oligosaccharides. In the probiotic diets, BacPack ABF™ was included at the expense of corn.
ME = metabolizable energy.
NE = net energy.
STTD = standardized total tract digestible.
+/- Inclusion rate of BacPack ABF™ in the probiotic diet in nursery was 0.05%.

Table 3. Chemical analysis of experimental diets (as-fed basis)[1]

	Sow diets			Nursery diets					
		Lactation		Phase 1		Phase 2		Phase 3	
Item	Gestation	Control	Probiotic	Control	Probiotic	Control	Probiotic	Control	Probiotic
Proximate analysis, %[2]									
DM	88.1	88.9	88.7	91.3	91.1	89.7	89.6	88.4	88.1
CP	13.1	20.2	20.2	19.6	19.9	20.6	20.9	21.7	20.9
ADF	2.7	3.0	2.8	2.1	2.4	2.9	2.6	4.1	3.8
NDF	8.2	7.6	7.4	5.1	5.5	6.5	6.4	7.4	9.3
Fat	2.3	5.0	5.1	4.8	4.9	3.1	3.0	3.3	2.8
Ca	1.30	1.05	1.12	1.11	1.05	0.90	0.92	0.92	0.95
P	0.64	0.63	0.64	0.69	0.73	0.69	0.68	0.60	0.60
Bacillus subtilis C-3102, CFU/g	*	3.0×10^3	1.1×10^6	1.3×10^4	4.0×10^5	3.4×10^4	5.0×10^5	5.2×10^4	5.4×10^5

[1]Diet samples were collected at manufacturing and composite samples were submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and quantification of *Bacillus subtilis* C-3102 (Calsporin®; Calpis Co. Ltd., Tokyo, Japan).

[2]DM = dry matter. CP = crude protein. ADF = acid detergent fiber. NDF = neutral detergent fiber.

**Bacillus subtilis* C-3102 in gestation top dress was 5.1×10^3 CFU/g in control and 2.2×10^7 CFU/g in probiotic.

Table 4. Effect of supplementation of Calsporin® (*Bacillus subtilis* C-3102) during gestation and lactation on sow and piglet performance until weaning[1]

	Control	Probiotic[2]	SEM	Probability, *P* =
Count, n	14	15	---	---
Parity	1.9	2.0	0.26	0.319
Sow BW, lb				
d 30 gestation	442.5	441.5	15.3	0.803
d 112 gestation	535.9	521.7	19.3	0.145
Post-farrow	493.0	482.7	17.0	0.218
Wean	485.4	478.4	17.0	0.366
Change, farrow to wean	-9.5	-4.3	4.1	0.377
Sow ADFI, lb				
Gestation[3]	5.2	5.2	0.19	0.944
Lactation	13.1	13.7	0.38	0.056
Lactation length, d	19.4	19.4	0.29	0.973
Total born, n	15.5	16.8	0.95	0.201
Born alive, n	14.1	14.5	0.72	0.624
Stillborn and mummy, n	1.4	2.3	0.59	0.228
Born alive, %*	90.9	86.1	2.18	0.135
Stillborn, %*	8.2	10.3	1.92	0.450
Piglet BW, lb				
Birth	3.12	3.05	0.11	0.664
d 2	3.65	3.44	0.13	0.276
d 12	8.55	8.67	0.30	0.755
Wean	12.65	12.90	0.46	0.601
Piglet ADG, lb	0.49	0.51	0.02	0.316
Litter weight, lb				
Birth	44.3	43.4	2.57	0.722
d 2	48.8	47.4	2.02	0.626
d 12	108.5	110.8	4.78	0.730
Wean	160.1	162.8	6.79	0.755
Litter size, n				
d 2[4]	13.3	13.8	0.24	0.060
d 12	12.6	12.8	0.31	0.719
Wean	12.7	12.7	0.32	0.916
Pre-wean mortality, %[5,*]	10.5	12.4	2.24	0.557

[1]A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a 100-d trial. Dietary treatments were fed to sows from d 30 of gestation until weaning on d 19 of lactation.
[2]Probiotic diet was supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of complete feed in gestation and 1,000,000 CFU/g of complete feed in lactation.
[3]Feed allowance in gestation was 4.5, 5.5, or 6.5 lb per day according to sow body condition.
[4]Cross-fostering was performed within treatments in an attempt to equalize litter size.
[5]Percent pre-wean mortality = mortality count from birth to wean ÷ born alive
*Variables analyzed using a binomial distribution.
ADG = average daily gain. ADFI = average daily feed intake.

Table 5. Effects of supplementation of Calsporin® (*Bacillus subtilis* C-3102) during gestation and lactation on nursing piglet fecal microflora[1, 2]

	d 2 Lactation		d 18 Lactation		Probability, *P* =		
Bacteria[4]	Control	Probiotic[3]	Control	Probiotic[3]	Treatment × day	Treatment	Day
Bacillus subtilis C-3102	2.44[b]	2.95[b]	2.51[b]	5.39[a]	<0.001	<0.001	<0.001
SEM	0.36	0.35	0.22	0.21			
Detected/sampled	5/13	9/14	7/13	14/14			
Total *Bacillus* sp.	5.83[ab]	6.28[a]	3.39[c]	5.41[b]	0.007	<0.001	<0.001
SEM	0.30	0.29	0.20	0.19			
Detected/sampled	13/13	14/14	11/13	14/14			
Lactobacillus sp.	6.91[b]	7.84[ab]	8.38[a]	8.06[a]	0.030	0.342	0.005
SEM	0.41	0.40	0.12	0.12			
Detected/sampled	12/13	14/14	13/13	14/14			
Clostridium perfringens	8.83	9.02	8.53	8.60	0.750	0.484	0.063
SEM	0.18	0.17	0.20	0.19			
Detected/sampled	13/13	14/14	13/13	14/14			
Enterococcus sp.	9.70	9.92	9.74	9.64	0.156	0.583	0.267
SEM	0.13	0.13	0.08	0.08			
Detected/sampled	13/13	14/14	13/13	14/14			
Enterobacteriaceae	9.33	9.28	8.35	8.40	0.623	0.983	<0.001
SEM	0.11	0.10	0.14	0.13			
Detected/sampled	13/13	14/14	13/13	14/14			
Total aerobes	8.24	8.23	6.77	6.64	0.849	0.810	<0.001
SEM	0.15	0.14	0.43	0.41			
Detected/sampled	13/13	14/14	13/13	14/14			
Total anaerobes	9.42	9.44	8.64	8.57	0.691	0.803	<0.001
SEM	0.11	0.10	0.12	0.12			
Detected/sampled	13/13	14/14	13/13	14/14			

[1]A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a 100-d trial. Dietary treatments were fed to sows from d 30 of gestation until weaning on d 19 of lactation. Fecal samples representing the litter were collected directly from the rectum of piglets on days 2 and 18 of lactation.

[2]Units are $\log_{10}$ CFU/g.

[3]Probiotic diet was supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of complete feed in gestation and 1,000,000 CFU/g of complete feed in lactation.

[4]Limit of detection was 2×10^2 CFU/g. *Salmonella* spp. was detected on d 2 of lactation in 1/13 fecal samples from litters from control-fed sows (7.33 $\log_{10}$ CFU/g), but it was not detectable on d 18 of lactation.

[ab]Indicate significant difference ($P < 0.05$) in the row.

Table 6. Effects of supplementation of Calsporin® (*Bacillus subtilis* C-3102) during gestation and lactation on sow fecal microflora[1, 2]

	d 30 Gestation		d 113 Gestation		d 18 Lactation		Probability, *P* =		
Bacteria[4]	Control	Probiotic[3]	Control	Probiotic[3]	Control	Probiotic[3]	Treatment × day	Treatment	Day
Bacillus subtilis C-3102	3.13[c]	4.69[b]	1.76[d]	6.14[a]	2.69[c]	6.20[a]	0.0034	<0.001	0.0314
SEM	0.39	0.37	0.17	0.16	0.19	0.18			
Detected/sampled	8/10	10/10	2/14	15/15	9/14	15/15			
Total *Bacillus* sp.	4.86[c]	5.32[b]	4.86[c]	6.16[a]	4.25[d]	6.22[a]	<0.001	<0.001	<0.001
SEM	0.11	0.10	0.05	0.05	0.05	0.05			
Detected/sampled	10/10	10/10	14/14	15/15	14/14	15/15			
Lactobacillus sp.	7.09	7.17	7.38	6.30	8.52	8.37	0.109	0.184	<0.001
SEM	0.22	0.21	0.41	0.40	0.17	0.17			
Detected/sampled	10/10	10/10	14/14	13/15	14/14	15/15			
Clostridium perfringens	8.06	8.01	7.93	7.55	6.14	6.02	0.351	0.196	<0.001
SEM	0.08	0.07	0.13	0.13	0.24	0.23			
Detected/sampled	10/10	10/10	14/14	15/15	14/14	15/15			
Enterobacteriaceae	7.41	7.56	7.30	7.43	6.69	6.45	0.411	0.951	<0.001
SEM	0.19	0.18	0.16	0.16	0.25	0.24			
Detected/sampled	10/10	10/10	14/14	15/15	14/14	15/15			
Total aerobes	8.23	8.60	8.32	8.32	8.69	8.38	0.117	0.869	0.368
SEM	0.17	0.16	0.16	0.16	0.14	0.13			
Detected/sampled	10/10	10/10	14/14	15/15	14/14	15/15			
Total anaerobes	9.11	9.20	9.07	8.92	9.35	9.25	0.250	0.437	0.001
SEM	0.09	0.08	0.08	0.08	0.07	0.06			
Detected/sampled	10/10	10/10	14/14	15/15	14/14	15/15			

[1]A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a 100-d trial. Dietary treatments were fed to sows from d 30 of gestation until weaning on d 19 of lactation. Fecal samples were collected directly from the rectum of sows on days 30 of gestation (baseline), 113 of gestation (pre-farrowing), and 18 of lactation (pre-weaning).

[2]Units are $\log_{10}$ CFU/g.

[3]Probiotic diet was supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of complete feed in gestation and 1,000,000 CFU/g of complete feed in lactation.

[4]Limit of detection was 2×10^2 CFU/g. *Salmonella* spp. was detected on d 113 of gestation in 2/14 fecal samples from control-fed sows (average 5.49 $\log_{10}$ CFU/g) and in 1/15 fecal samples from probiotic-fed sows (4.34 $\log_{10}$ CFU/g), but it was not detectable on d 30 of gestation and d 18 of lactation. *Enterococcus* sp. was not analyzed in sow fecal samples.

[abcd]Indicate significant difference ($P < 0.05$) in the row.

Table 7. Interactive effects of sow and nursery pig dietary treatment on growth performance of nursery pigs[1]

Sow treatment[2]:	Control		Probiotic			Probability, *P* =		
Nursery treatment[3]:	Control	Probiotic	Control	Probiotic	SEM	Sow treatment × nursery treatment	Sow treatment	Nursery treatment
d 0 to 7								
ADG, lb	0.14	0.18	0.14	0.15	0.017	0.333	0.418	0.084
ADFI, lb	0.25	0.26	0.26	0.26	0.016	0.853	0.704	0.681
F/G	2.38	1.58	2.18	1.40	0.831	0.984	0.820	0.341
d 7 to 21								
ADG, lb	0.69	0.70	0.71	0.68	0.022	0.359	0.986	0.560
ADFI, lb	0.96	0.98	0.99	0.94	0.026	0.151	0.959	0.549
F/G	1.40	1.41	1.40	1.39	0.024	0.608	0.678	0.920
d 21 to 42								
ADG, lb	1.38	1.35	1.31	1.30	0.022	0.628	0.005	0.293
ADFI, lb	2.05	2.04	1.95	1.94	0.036	0.980	0.008	0.702
F/G	1.48	1.51	1.49	1.50	0.009	0.264	0.648	0.088
d 0 to 42								
ADG, lb	0.94	0.93	0.91	0.90	0.018	0.755	0.135	0.535
ADFI, lb	1.37	1.38	1.35	1.32	0.027	0.595	0.146	0.661
F/G	1.47	1.48	1.48	1.48	0.008	0.467	0.994	0.518
BW, lb								
d 0	12.9	12.9	13.0	13.0	0.02	0.995	<0.001	0.547
d 7	13.8	14.1	14.0	14.0	0.12	0.350	0.940	0.114
d 21	23.8	24.0	23.9	23.5	0.38	0.441	0.677	0.795
d 42	52.8	52.7	51.5	51.0	0.74	0.841	0.042	0.707

[1]A total of 358 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 12.9 lb were used in a 42-d nursery trial with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were weaned at approximately 20 d of age and allotted to treatments in a completely randomized design. Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control or probiotic) and nursery pig treatment (control or probiotic).

[2]Sow treatment consisted of providing a control diet or a probiotic diet supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g in gestation (d 30 to farrowing) and 1,000,000 CFU/g in lactation (farrowing to weaning).

[3]Nursery treatment consisted of providing a control diet or a probiotic diet supplemented with BacPack ABF™ (*Bacillus subtilis* C-3102, beta glucans, and mannan oligosaccharides; Quality Technology International, Inc., Elgin, IL) at 0.05% inclusion.

ADG = average daily gain. ADFI = average daily feed intake. F/G = feed-to-gain ratio.

Table 8. Main effects of sow and nursery pig dietary treatment on growth performance of nursery pigs[1]

	Sow treatment[2]			Probability,	Nursery treatment[3]			Probability,
Item[4]	Control	Probiotic	SEM	*P* =	Control	Probiotic	SEM	*P* =
d 0 to 7								
ADG, lb	0.16	0.15	0.012	0.418	0.14	0.17	0.012	0.084
ADFI, lb	0.25	0.26	0.012	0.704	0.25	0.26	0.012	0.681
F/G	1.98	1.79	0.588	0.820	2.28	1.49	0.588	0.341
d 7 to 21								
ADG, lb	0.70	0.70	0.016	0.986	0.70	0.69	0.016	0.560
ADFI, lb	0.97	0.97	0.018	0.959	0.98	0.96	0.018	0.549
F/G	1.41	1.40	0.017	0.678	1.40	1.40	0.017	0.920
d 21 to 42								
ADG, lb	1.37	1.30	0.015	0.005	1.35	1.32	0.015	0.293
ADFI, lb	2.04	1.95	0.026	0.008	2.00	1.99	0.026	0.702
F/G	1.50	1.49	0.007	0.648	1.49	1.50	0.007	0.088
d 0 to 42								
ADG, lb	0.93	0.90	0.013	0.135	0.92	0.91	0.013	0.535
ADFI, lb	1.38	1.34	0.019	0.146	1.36	1.35	0.019	0.661
F/G	1.48	1.48	0.006	0.994	1.47	1.48	0.006	0.518
BW, lb								
d 0	12.9	13.0	0.01	<0.001	12.9	12.9	0.01	0.547
d 7	14.0	14.0	0.08	0.940	13.9	14.1	0.08	0.114
d 21	23.9	23.7	0.27	0.677	23.9	23.8	0.27	0.795
d 42	52.8	51.2	0.53	0.042	52.1	51.9	0.53	0.707

[1]A total of 358 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 12.9 lb were used in a 42-d nursery trial with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were weaned at approximately 20 d of age and allotted to treatments in a completely randomized design. Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control or probiotic) and nursery pig treatment (control or probiotic).

[2]Sow treatment consisted of providing a control diet or a probiotic diet supplemented with Calsporin® (*Bacillus subtilis* C-3102; Quality Technology International, Inc., Elgin, IL) to achieve 500,000 CFU/g in gestation (d 30 to farrowing) and 1,000,000 CFU/g in lactation (farrowing to weaning).

[3]Nursery treatment consisted of providing a control diet or a probiotic diet supplemented with BacPack ABF™ (*Bacillus subtilis* C-3102, beta glucans, and mannan oligosaccharides; Quality Technology International, Inc., Elgin, IL) at 0.05% inclusion.

[4]ADG = average daily gain. ADFI = average daily feed intake. F/G = feed-to-gain ratio.

Table 9. Effects of sow and nursery pig dietary treatment on fecal microflora of nursery pigs[1,2]

	d 21 Nursery				d 42 Nursery			
Sow treatment[3]	Control		Probiotic		Control		Probiotic	
Nursery treatment[4]	Control	Probiotic	Control	Probiotic	Control	Probiotic	Control	Probiotic
Bacteria[5]								
Bacillus subtilis C-3102	2.67	5.57	3.38	5.52	3.54	5.81	2.45	5.75
SEM	0.20	0.20	0.20	0.20	0.25	0.25	0.25	0.25
Detected/sampled	4/6	6/6	6/6	6/6	6/6	6/6	3/6	6/6
Total *Bacillus* sp.	3.96	5.60	4.09	5.55	4.11	5.85	4.18	5.78
SEM	0.16	0.16	0.16	0.16	0.09	0.09	0.09	0.09
Detected/sampled	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Lactobacillus sp.	9.14	9.05	8.90	9.12	8.94	8.69	8.96	8.85
SEM	0.09	0.09	0.09	0.09	0.11	0.11	0.11	0.11
Detected/sampled	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Enterococcus sp.	3.97	4.23	4.05	4.45	4.47	4.76	4.94	5.13
SEM	0.53	0.53	0.53	0.53	0.52	0.52	0.52	0.52
Detected/sampled	6/6	5/6	6/6	5/6	6/6	5/6	6/6	6/6
Enterobacteriaceae	7.58	6.71	7.22	7.57	7.49	7.44	7.26	7.43
SEM	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23
Detected/sampled	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Total aerobes	9.62	9.64	9.42	9.53	9.76	9.59	9.65	9.55
SEM	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Detected/sampled	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Total anaerobes	10.25	10.13	10.14	10.22	10.21	10.09	10.05	10.13
SEM	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05
Detected/sampled	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6

[1]A total of 358 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 12.9 lb were used in a 42-d nursery trial. Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control or probiotic) and nursery pig treatment (control or probiotic). Fecal samples were collected directly from the rectum of pigs on d 21 and 42 of nursery. Samples were collected from two pigs per pen and three pens of the same treatment were pooled for microbial analysis (n = 24).

[2]Units are $\log_{10}$ CFU/g. Probability (*P*-values) are shown in Table 10.

[3]Sow treatment consisted of providing a control diet or a probiotic diet supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g in gestation (d 30 to farrowing) and 1,000,000 CFU/g in lactation (farrowing to weaning).

[4]Nursery treatment consisted of providing a control diet or a probiotic diet supplemented with BacPack ABF™ (*Bacillus subtilis* C-3102, beta glucans, and mannan oligosaccharides; Quality Technology International, Inc., Elgin, IL) at 0.05% inclusion.

[5]Limit of detection was 2×10^2 CFU/g. *Clostridium perfringens* and *Salmonella* spp. were not detectable on d 21 and 42 of nursery.

Table 10. Probability of main effects and interactions of sow treatment, nursery treatment, and day on fecal microflora of nursery pigs[1,2,3]

Bacteria	Sow treatment × nursery treatment × day	Sow treatment × nursery treatment	Sow treatment × day	Nursery treatment × day	Sow treatment	Nursery treatment	Day
Bacillus subtilis C-3102	0.009	0.695	0.009	0.399	0.460	<0.001	0.509
Total *Bacillus* sp.	0.912	0.337	0.832	0.525	0.824	<0.001	0.082
Lactobacillus sp.	0.538	0.146	0.223	0.090	0.974	0.443	0.012
Enterococcus sp.	0.862	0.979	0.689	0.897	0.486	0.487	0.068
Enterobacteriaceae	0.122	0.057	0.230	0.300	0.716	0.578	0.379
Total aerobes	0.931	0.419	0.372	0.036	0.022	0.407	0.071
Total anaerobes	0.868	0.012	0.383	0.999	0.364	0.568	0.039

[1]A total of 358 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 12.9 lb were used in a 42-d nursery trial. Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control or probiotic) and nursery pig treatment (control or probiotic). Fecal samples were collected for microbial analysis on d 21 and 42 of nursery and analyzed as repeated measures of day within experimental unit.
[2]Sow treatment consisted of providing a control diet or a probiotic diet supplemented with Calsporin® (*Bacillus subtilis* C-3102; Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g in gestation (d 30 to farrowing) and 1,000,000 CFU/g in lactation (farrowing to weaning).
[3]Nursery treatment consisted of providing a control diet or a probiotic diet supplemented with BacPack ABF™ (*Bacillus subtilis* C-3102, beta glucans, and mannan oligosaccharides; Quality Technology International, Inc., Elgin, IL) at 0.05% inclusion.

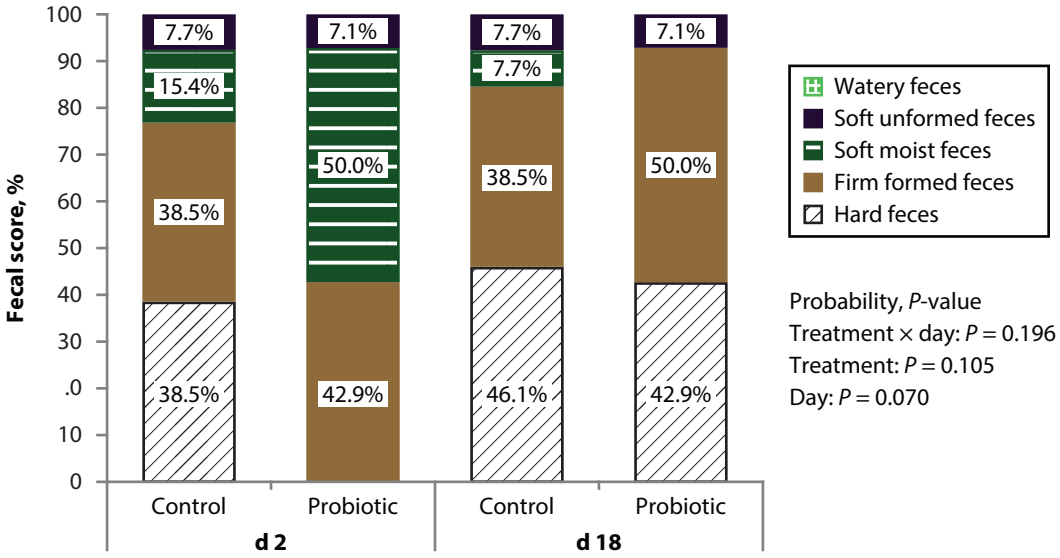

Figure 1. Effect of supplementation of Calsporin® (*Bacillus subtilis* C-3102) during gestation and lactation on piglet fecal scoring during the nursing phase (n = 27 litters).

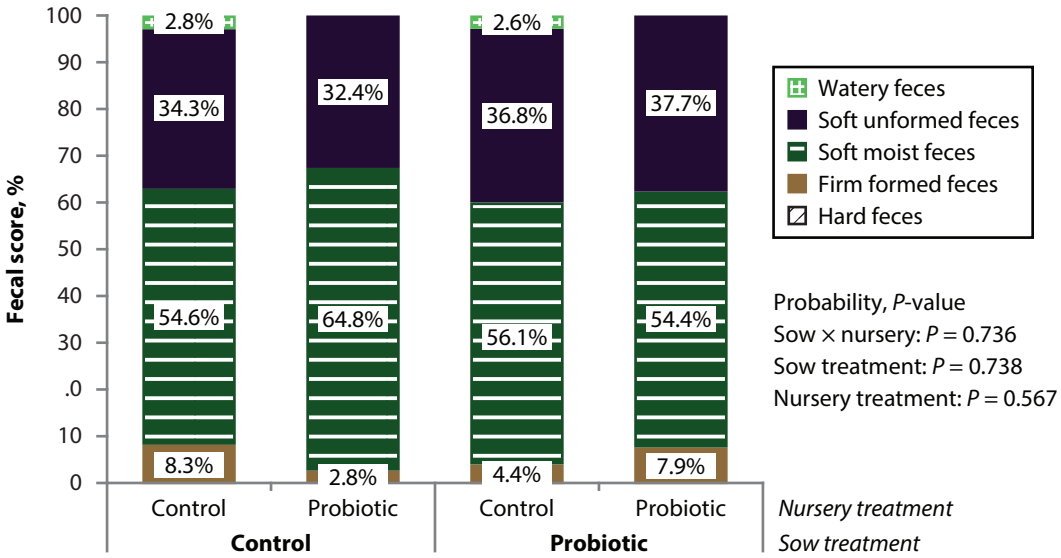

Figure 2. Effects of sow and nursery pig dietary treatment on fecal score of nursery pigs (n = 74 pens).

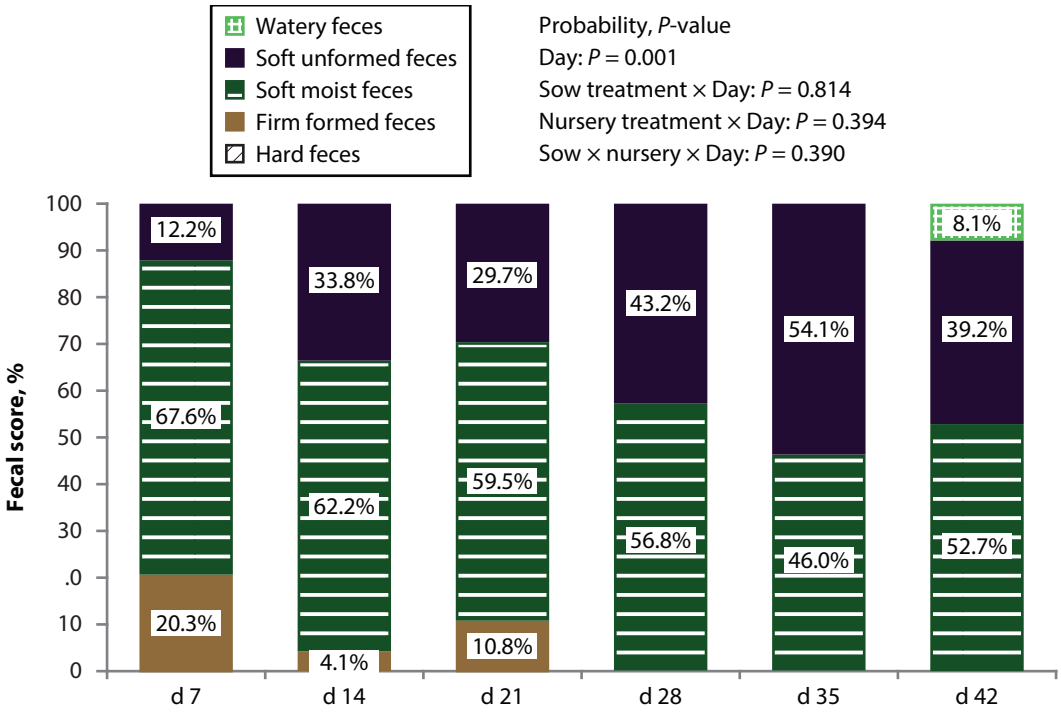

Figure 3. Effects of days into the nursery on fecal score of nursery pigs (n = 74 pens).