# Effects of *Bacillus subtilis* C-3102 on sow and progeny performance, fecal consistency, and fecal microbes during gestation, lactation, and nursery periods[1,2]

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**ABSTRACT:** This study evaluated the effects of providing a dietary probiotic, *Bacillus subtilis* C-3102, to sows during gestation and lactation and to progeny after weaning on performance, fecal consistency, and fecal microbes. For the sow portion of the study, 29 sows and litters were used from day 30 of gestation until weaning. Sow treatments consisted of control diet or probiotic diet with *B. subtilis* C-3102 at 500,000 cfu/g of gestation feed and 1,000,000 cfu/g of lactation feed. For the nursery portion of the study, 358 weaned pigs, progeny of sows on study, were used in a 42-d nursery study. Nursery treatments consisted of control diet or probiotic diet with *B. subtilis* C-3102 and prebiotics at 500,000 cfu/g of nursery feed. Treatments were arranged in a split-plot design with sow treatment (control or probiotic diet) as main plot and nursery treatment (control or probiotic diet) as subplot. Performance, fecal consistency by fecal score method, and fecal microbes by isolation and enumeration method were assessed. In lactation, probiotic-fed sows tended ($P = 0.057$) to have increased feed intake, but it did not improve ($P > 0.05$) sow or litter performance in lactation. In the nursery, there were no ($P > 0.10$) interactions or main effects of sow or nursery treatments on overall growth performance. However, pigs born from control-fed sows had greater ($P < 0.05$) average daily gain, average daily feed intake, and body weight in late nursery than pigs born from probiotic-fed sows. Fecal score evaluation of nursing and nursery pigs indicated no influence ($P > 0.05$) of sow or nursery treatments on fecal consistency. Fecal microbial analysis revealed a modest modification in fecal microbial population by increasing ($P < 0.05$) the number of total *Bacillus* sp. in probiotic-fed sows and nursery pigs. Nursing piglets born from probiotic-fed sows carried over ($P < 0.05$) this modification in fecal microbial population preweaning. In conclusion, providing a probiotic based on *B. subtilis* C-3102 to sows during gestation and lactation and to progeny after weaning did not elicit noteworthy improvements in performance or fecal consistency, but there was a benefit on sow lactation feed intake. Fecal microbial analysis indicated a maternal-progeny intestinal microbiota relationship with pigs born from probiotic-fed sows displaying similar fecal microbial population as sows. However, pigs born from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery.

**Key words:** direct-fed microbial, feed additive, microbiota, probiotic, swine



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## INTRODUCTION

Probiotics have been explored as feed additives in swine diets to improve performance and preserve intestinal health while minimizing the use of antibiotics (Liao and Nyachoti, 2017). The use of probiotics in sow diets is proposed to have a dual purpose benefiting sows and their progeny. The intimate maternal contact is an important determinant of gastrointestinal tract bacterial colonization of newborn piglets (Everaert et al., 2017). Moreover, sows are able to exert a diet-driven modulation of milk bacterial population and influence the progeny intestinal microbiota during lactation (Rodriguez, 2014; Chen et al., 2018). The establishment of a healthy intestinal microbiota in early life may be essential to promote growth, immunity, and health later in life (Schmidt et al., 2011; Merrifield et al., 2016; Dou et al., 2017). Thus, dietary strategies to modulate the intestinal microbiota of sows and piglets have been investigated. Studies have demonstrated that provision of probiotics to sows can modify the sow fecal microbial population and carry over to progeny in preweaning and postweaning stages (Silva et al., 2010; Baker et al., 2013; Starke et al., 2013).

*Bacillus subtilis* C-3102 is a nongenetically modified strain of a gram-positive, spore-forming bacteria used as probiotic for pigs. The effects of *B. subtilis* C-3102 are proposed to promote beneficial bacteria proliferation in sows and reduce pathogenic bacteria in their progeny (Maruta et al., 1996b; Kritas et al., 2015). This has been reflected as reduction in diarrhea incidence (Maruta et al., 1996b), improvement in growth performance (Marubashi et al., 2012), and attenuation of intestinal lesions under health challenge (Canning et al., 2017) in nursery pigs. However, to the best of the authors' knowledge, studies designed to evaluate the long-term influence of providing *B. subtilis* C-3102 to sows in gestation and lactation on the progeny through the nursery have not been previously conducted.

Therefore, the objective of this study was to evaluate the effects of providing a probiotic based on viable spores of *B. subtilis* C-3102 to sows during gestation and lactation and to progeny after weaning on performance, fecal consistency, and fecal microbes.

## MATERIALS AND METHODS

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. Sows and progeny were used in this study divided in sow portion from day 30 of gestation to weaning and nursery portion from weaning to day 42.

### *Sow Portion*

A total of 29 crossbred sows (DNA 241, DNA Genetics, Columbus, NE; 1.9 average parity) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used for the sow portion of the study. Sows were individually housed in environmentally controlled and mechanically ventilated barns during gestation and lactation. Farrowing stalls were equipped with an individual nipple waterer and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada). Farrowing stalls were equipped with a rubber mat and heat lamp for piglet comfort. Piglets were processed and cross-fostered within sow treatment group to equalize litter size within 24 h of birth. Piglets had free access to water and no creep feeding was provided during lactation.

Dietary treatments were assigned to sows with confirmed pregnancy on day 30 of gestation in a randomized complete block design based on sow parity and initial body weight (**BW**). Sow dietary treatments consisted of providing a control diet ($n = 14$ sows) or a probiotic diet ($n = 15$ sows) to sows during gestation and lactation. The probiotic diet was supplemented with a probiotic product based on viable spores of *B. subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan). The active ingredient in Calsporin is dried *B. subtilis* C-3102 fermentation product in a calcium carbonate carrier. Diets were based on corn and soybean meal and fed in meal form (Table 1). Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Gestation diets were fed from day 30 of gestation until farrowing. Daily feed allowance was 2, 2.5, or 3 kg once per day according to body condition from day 30 to 112 of gestation and 2.7 kg/d from day 112 of gestation until farrowing. Dietary 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 *B. subtilis* C-3102 in gestation feed at the expense of corn.

Lactation diets were fed from farrowing to weaning at approximately day 19 of lactation. Sows were allowed ad libitum feed intake during lactation

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

| Item | Gestation | Lactation |
|---|---|---|
| Ingredient, % | | |
| Corn | 80.7 | 63.4 |
| Soybean meal, 47% crude protein | 15.6 | 30.6 |
| Choice white grease | — | 2.50 |
| Calcium carbonate | 1.15 | 0.90 |
| Monocalcium phosphate, 21.5% phosphorus | 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[2] | 0.50 | 0.50 |
| Trace mineral premix[3] | 0.15 | 0.15 |
| Phytase[4] | 0.02 | 0.02 |
| Probiotic[5] | +/− | +/− |
| Total | 100.0 | 100.0 |
| Calculated analysis | | |
| SID[6] 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 |
| Net energy, kcal/kg | 2,476 | 2,524 |
| Crude protein, % | 14.1 | 20.1 |
| Calcium, % | 0.85 | 0.75 |
| STTD[7] phosphorus, % | 0.48 | 0.44 |

[1]Gestation diets were fed from day 30 of gestation until farrowing and lactation diets were fed from farrowing until weaning on day 19 of lactation. Diets were fed in meal form.

[2]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 22,455 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin $B_{12}$; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 88 mg biotin; 661 mg folic acid; 1,984 mg pyridoxine; 220,460 mg choline; and 19,841 mg carnitine.

[3]Provided per kg of premix: 73 g Zn from zinc sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite; 0.08 g chromium picolinate.

[4]Ronozyme HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ) provided 405 FTU/kg of feed.

[5]Calsporin 1.0B (Calpis Co. Ltd., Tokyo, Japan) provided viable spores of *Bacillus subtilis* C-3102 at $1 \times 10^9$ cfu/g of product. In gestation, it was top dressed in probiotic diets to achieve 500,000 cfu/g of feed. In lactation, it was included in probiotic diets to achieve 1,000,000 cfu/g of feed (0.10% inclusion rate). Calsporin 1.0B was included in the diets at the expense of corn.

+/− indicates inclusion in probiotic diets and absence of inclusion in control diets.

[6]SID = standardized ileal digestible.

[7]STTD = standardized total tract digestible.

with daily feed delivery and recording by an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada). Dietary treatments were incorporated into lactation diet formulation. In the probiotic diet, Calsporin was included to achieve 1,000,000 cfu/g of *B. subtilis* C-3102 in lactation feed at the expense of corn.

Sow BW was measured on days 30 and 112 of gestation, postfarrow, and at weaning. Sow feed intake was recorded on a daily basis. Fecal samples were collected from sows on day 30 of gestation (baseline), day 112 of gestation (prefarrowing), and day 18 of lactation (preweaning) for microbial analysis. Farrowing and litter performance were assessed by recording number of total born piglets, born alive piglets, stillborn, and mummies; individual piglet BW at birth, days 2 and 12 of lactation, and at weaning; and litter size on days 2 and 12 of lactation and at weaning. Preweaning mortality was estimated considering the number of dead piglets from birth to weaning in relation to the number of piglets born alive. Fecal score was conducted to characterize piglet fecal consistency on day 2 (postnatal) and day 18 (preweaning). Fecal samples were collected from piglets on day 2 (postnatal) and day 18 (preweaning) for microbial analysis.

### *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 the study. Only nine weaned pigs (five from control litters and four from probiotic litters) were not included in the nursery portion of the study due to health issues. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d period into the nursery beginning at weaning. Weaned pigs were housed in an environmentally controlled and mechanically ventilated nursery barn with 1.5- × 1.5-m pens equipped with a four-hole, dry, self-feeder and one cup waterer. Pigs were placed in mixed-gender pens with 4 or 5 pigs per pen.

Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot. There were 18 or 19 replicates per treatment. Nursery dietary treatments consisted of providing

a control diet or a probiotic diet with supplementation of viable spores of *B. subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs. In the probiotic diet, BacPack ABF was included at 0.05% of complete feed to achieve 500,000 cfu/g of *B. subtilis* C-3102 and proprietary amounts of yeast cell wall derivatives. The active ingredients in BacPack ABF are dried *B. subtilis* C-3102 fermentation product and mannan oligosaccharides in a calcium carbonate carrier.

Diets were based on corn and soybean meal and fed in three dietary phases: phase 1, fed from day 0 to 7 in pellet form; phase 2, fed from day 7 to 21 in meal form; and phase 3, fed from day 21 to 42 in meal form (Table 2). Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Pigs and feeders were weighed on days 0, 7, 14, 21, 28, 35, and 42 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Fecal score was conducted to characterize piglet fecal consistency on days 0, 7, 14, 21, 28, 35, and 42. Fecal samples were collected from pigs on days 21 and 42 for microbial analysis.

### *Fecal Score*

Fecal score was conducted to categorize the piglet fecal consistency using the following categories: hard feces, firm formed feces, soft moist feces, soft unformed feces, and watery feces. Fecal scoring was assigned to litters in the sow portion of the study and to pens in the nursery portion of the study by visually assessing feces in farrowing stalls or nursery pens. Fecal score evaluation was performed by three trained individuals and the concordant score was considered as the definite score.

### *Fecal Microbial Analysis*

Fecal samples were freshly collected from sows by rectal grab, from nursing piglets with sterile mini cotton tip swabs, and from nursery pigs with sterile cotton tip swabs. In the sow portion of the study, fecal samples were collected from individual sows for analysis ($n$ = 29) and from all nursing piglets pooled by litter for analysis ($n$ = 27). Fecal samples from one litter of each sow treatment group were not collected for microbial analysis due to sows farrowing later compared to the group. In the nursery portion of the study, fecal samples were collected from two pigs per pen and three pens of the same treatment were pooled for analysis ($n$ = 24).

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

| Item | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| Ingredient, % | | | |
| Corn | 43.0 | 55.2 | 60.8 |
| Soybean meal, 47% crude protein | 18.8 | 24.8 | 34.6 |
| Whey powder, 11.5% crude protein | 25.0 | 10.0 | — |
| Fish meal, 63% crude protein | 4.50 | — | — |
| Enzyme-treated soybean meal[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% phosphorus | 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[3] | 0.25 | 0.25 | 0.25 |
| Trace mineral premix[4] | 0.15 | 0.15 | 0.15 |
| Vitamin E, 20,000 IU | 0.05 | — | — |
| Choline chloride 60% | 0.04 | — | — |
| Phytase[5] | 0.02 | 0.02 | 0.02 |
| Zinc oxide | 0.39 | 0.25 | — |
| Probiotic[6] | +/− | +/− | +/− |
| Total | 100.0 | 100.0 | 100.0 |
| Calculated analysis | | | |
| SID[7] 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 |
| Net energy, kcal/kg | 2,632 | 2,485 | 2,443 |
| Crude protein, % | 20.5 | 21.1 | 22.1 |
| Calcium, % | 0.75 | 0.70 | 0.70 |
| STTD[8] phosphorus, % | 0.49 | 0.43 | 0.36 |

[1]Nursery diets were fed in three dietary phases: phase 1, from day 0 to 7 after weaning in pellet form; phase 2, from day 7 to 21 in meal form; and phase 3, from day 21 to 42 in meal form.

[2]HP 300 (Hamlet Protein, Inc., Findlay, OH), 56% crude protein.

[3]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin $B_{12}$; 19,841 mg niacin; 11,023 mg pantothenic acid; and 3,307 mg riboflavin.

[4]Provided per kg of premix: 73 g Zn from zinc sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; and 0.2 g Se from sodium selenite.

[5] Ronozyme HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ) provided 405 FTU/kg of feed.

[6] BacPack ABF (Quality Technology International, Inc., Elgin, IL) provided viable spores of *Bacillus subtilis* C-3102 at $1 \times 10^9$ cfu/g of product and proprietary amounts of yeast cell wall derivatives. BacPack ABF was included in the diets to achieve 500,000 cfu/g of feed (0.05% inclusion rate) at the expense of corn.

+/− Indicates inclusion in probiotic diets and absence of inclusion in control diets.

[7]SID = standardized ileal digestible.

[8]STTD = standardized total tract digestible.

Fecal samples were kept at 4 °C until analysis within 24 h of collection.

Microbial analysis of fecal samples was performed by isolation and enumeration method of *B. subtilis* C-3102, total *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Clostridium perfringens*, *Salmonella* spp., Enterobacteriaceae, total aerobes, and total anaerobes.

For microbial platting, approximately 1 g of feces was suspended in 9 mL of anaerobic diluent and serial 10-fold dilutions were prepared according to procedures described previously (Maruta et al., 1996b). Aliquots of 0.05 mL of each dilution were inoculated into selective and nonselective media. All media were incubated at 37 °C unless otherwise noted. *Bacillus subtilis* C-3102 were enumerated on tryptic soy broth with 2% agar after incubation for 1 d (Marubashi et al., 2012). Total *Bacillus* sp. were enumerated by chromogenic method using a differential medium (92325 Bacillus *ChromoSelect* Agar, Sigma-Aldrich, Saint Louis, MO) after incubation for 1 d and spores were quantified after incubation at 80 °C for 15 min (Kritas et al., 2015). *Lactobacillus* sp. were enumerated on modified lactobacilli selective agar after anaerobic incubation for 2 d (Maruta et al., 1996b). *Enterococcus* sp. were enumerated on triphenyltetrazolium chloride-acridine orange-thallous sulfate aesculin crystal violet (**TATAC**) agar after incubation for 2 d (Maruta et al., 1996b). *Clostridium perfringens* were enumerated on neomycin-brilliant green-taurocholate-nagler (**NN**) agar after anaerobic incubation for 3 d (Maruta et al., 1996b). *Salmonella* spp. were enumerated on mannitol lysine crystal violet brilliant green (**MLCB**) agar after incubation for 1 d (Maruta et al., 1996a). Enterobacteriaceae were enumerated on neomycin-brilliant green-taurocholate-blood (**NBGT**) agar after incubation for 1 d (Maruta et al., 1996b). Total aerobes were enumerated on trypticase soy agar after incubation for 2 d (Maruta et al., 1996b). Total anaerobes were enumerated on glucose blood liver agar and Eggerth–Gagnon agar after anaerobe incubation for 3 d (Maruta et al., 1996b). Limit of detection was $2 \times 10^2$ cfu/g. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc. (Peachtree City, GA).

### *Chemical analysis*

Feed samples were collected during the manufacturing process. Approximately 1 kg of feed was collected from each treatment for each batch of feed. Composite samples were stored at −20 °C and grinded before submission to analysis. Feed samples were analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (method 935.29; AOAC, 1990), crude protein (method 990.03; AOAC, 1990), acid detergent fiber (Ankom Technology, 1998a), neutral detergent fiber (Ankom Technology, 1998b), ether extract (Ankom Technology, 2004), Ca (method 985.01; AOAC, 1990), and P (method 985.01; AOAC, 1990). Feed samples were also analyzed for quantification of *B. subtilis* C-3102 (Calpis America, Inc., Peachtree City, GA).

### *Statistical Analysis*

Data were analyzed using a linear mixed model. Dietary treatment was included as fixed effect. Block was included as random effect in the sow portion analysis of the study. The experimental units were sow or litter for the sow portion of the study and pen for the nursery portion of the study.

Response variables were fit assuming a normal distribution unless otherwise noted. Piglets born alive, stillborn, and mummies were analyzed assuming a binomial distribution as a proportion of total born piglets. Preweaning mortality was analyzed assuming a binomial distribution as a proportion of number of dead piglets from birth to weaning in relation to the number of piglets born alive. Fecal score was analyzed assuming a multinomial distribution and considering the frequency distribution of experimental units within each fecal score category. For normally distributed response variables, the residual assumptions were met by evaluating studentized residuals.

In the nursery portion of this study, preplanned contrast statements were built to evaluate the main effects and interactions of sow dietary treatment and nursery dietary treatment. Repeated measures analysis was applied to fecal score and fecal microbial analysis considering the multiple measures taken on the same experimental unit over a time period.

Statistical models were fit using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

## RESULTS

### *Chemical Analysis*

Proximate analysis, Ca, P, and *B. subtilis* C-3102 content of experimental diets (Tables 3 and 4) were consistent with formulated estimates. The

presence of *B. subtilis* C-3102 in control diets is associated to the ubiquitous nature of the species and was as expected. The levels in control diets were within expectations and in accordance to the literature (Marubashi et al., 2012), that is, at least 1 $\log_{10}$ lower cfu/g compared to probiotic diets.

### *Sow Portion*

There was no evidence for differences ($P > 0.10$) in sow parity and BW on day 30 of gestation between dietary treatments (Table 5), validating the randomization process. No evidence for differences ($P > 0.10$) was observed on sow BW at the end of gestation, postfarrow, or at weaning; consequently, no evidence for differences ($P > 0.10$) was observed on sow BW change from farrow to weaning between control- and probiotic-fed sows. In gestation, ADFI was similar ($P > 0.10$) for control- and probiotic-fed sows. In lactation, probiotic-fed sows had a tendency ($P = 0.057$) for increased ADFI, consuming on average 0.5 kg more feed per day in lactation than control-fed sows. There was no evidence for differences ($P > 0.10$) in number of piglets total born, born alive, stillborn, and mummies; piglet BW from birth to weaning; litter weight from birth to weaning; piglet ADG during lactation; and preweaning mortality between control- and probiotic-fed sows. Probiotic-fed sows had a tendency ($P = 0.060$) for larger litter size on day 2 after birth, with on average 0.5 more piglet per litter than control-fed sows. There was no evidence for differences ($P > 0.10$) in litter size on day 12 of lactation and at weaning between control- and probiotic-fed sows.

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

| | | Lactation | |
|---|---|---|---|
| Item | Gestation | Control | Probiotic |
| Proximate analysis, % | | | |
| Dry matter | 88.1 | 88.9 | 88.7 |
| Crude protein | 13.1 | 20.2 | 20.2 |
| Acid detergent fiber | 2.7 | 3.0 | 2.8 |
| Neutral detergent fiber | 8.2 | 7.6 | 7.4 |
| Ether extract | 2.3 | 5.0 | 5.1 |
| Calcium | 1.30 | 1.05 | 1.12 |
| Phosphorus | 0.64 | 0.63 | 0.64 |
| *Bacillus subtilis* C-3102, cfu/g | * | $3.0 \times 10^3$ | $1.1 \times 10^6$ |

[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 (Calpis America, Inc., Peachtree City, GA).

*Top dress analysis contained *Bacillus subtilis* C-3102 at $5.1 \times 10^3$ cfu/g of control top dress and $2.2 \times 10^7$ cfu/g of probiotic top dress.

### *Nursery Portion*

Only a tendency ($P < 0.10$) for interaction of sow dietary treatment and nursery dietary treatment was observed on growth performance of nursery pigs (Table 6). Therefore, the main effects of sow dietary treatment and nursery dietary treatment on growth performance of nursery pigs were further explored (Table 7).

Initial BW in the nursery was influenced ($P < 0.01$) by sow dietary treatment, where pigs born from probiotic-fed sows were 0.1 kg heavier than pigs born from control-fed sows. The difference in initial nursery BW was expected from the same difference in piglet weaning weight and as a consequence of split-plot design used in this study. The significance was captured in the nursery due to the greater number of replicates in the nursery portion of the study ($n = 36$ or 38 pens per treatment) compared to the sow portion of the study ($n = 14$ or 15 litters per treatment), in addition to the considerably lower variation around pig BW in the nursery portion of the study (initial

**Table 4.** Chemical analysis of nursery diets (as-fed basis)[1]

| | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|
| Item | Control | Probiotic | Control | Probiotic | Control | Probiotic |
| Proximate analysis, % | | | | | | |
| Dry matter | 91.3 | 91.1 | 89.7 | 89.6 | 88.4 | 88.1 |
| Crude protein | 19.6 | 19.9 | 20.6 | 20.9 | 21.7 | 20.9 |
| Acid detergent fiber | 2.1 | 2.4 | 2.9 | 2.6 | 4.1 | 3.8 |
| Neutral detergent fiber | 5.1 | 5.5 | 6.5 | 6.4 | 7.4 | 9.3 |
| Ether extract | 4.8 | 4.9 | 3.1 | 3.0 | 3.3 | 2.8 |
| Calcium | 1.11 | 1.05 | 0.90 | 0.92 | 0.92 | 0.95 |
| Phosphorus | 0.69 | 0.73 | 0.69 | 0.68 | 0.60 | 0.60 |
| *Bacillus subtilis* C-3102, cfu/g | $1.3 \times 10^4$ | $4.0 \times 10^5$ | $3.4 \times 10^4$ | $5.0 \times 10^5$ | $5.2 \times 10^4$ | $5.4 \times 10^5$ |

[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 (Calpis America, Inc., Peachtree City, GA).

**Table 5.** Effect of providing *Bacillus subtilis* C-3102 during gestation and lactation on sow and piglet performance until weaning[1]

| Item | Control | Probiotic[2] | SEM | Probability, $P <$ |
|---|---|---|---|---|
| Count, *n* | 14 | 15 | — | — |
| Parity | 1.9 | 2.0 | 0.26 | 0.319 |
| Gestation length, d | 115.3 | 115.2 | 0.23 | 0.787 |
| Lactation length, d | 19.4 | 19.4 | 0.29 | 0.973 |
| Sow BW, kg | | | | |
| Day 30 gestation | 200.7 | 200.2 | 6.94 | 0.803 |
| Day 112 gestation | 243.1 | 236.6 | 8.76 | 0.145 |
| Postfarrow | 223.6 | 218.9 | 7.74 | 0.218 |
| Wean | 220.2 | 217.0 | 7.69 | 0.366 |
| Change, farrow to wean | −4.3 | -1.9 | 1.87 | 0.377 |
| Sow ADFI, kg | | | | |
| Gestation | 2.4 | 2.4 | 0.09 | 0.944 |
| Lactation | 5.7 | 6.2 | 0.24 | 0.057 |
| 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, kg | | | | |
| Birth | 1.41 | 1.38 | 0.05 | 0.664 |
| Day 2 | 1.65 | 1.56 | 0.06 | 0.276 |
| Day 12 | 3.88 | 3.93 | 0.14 | 0.755 |
| Wean | 5.74 | 5.85 | 0.21 | 0.601 |
| Piglet ADG, g | 222 | 231 | 9.79 | 0.316 |
| Litter weight, kg | | | | |
| Birth | 20.1 | 19.7 | 1.16 | 0.722 |
| Day 2 | 22.1 | 21.5 | 0.91 | 0.626 |
| Day 12 | 49.2 | 50.3 | 2.17 | 0.730 |
| Wean | 72.6 | 73.8 | 3.08 | 0.755 |
| Litter size, *n* | | | | |
| Day 2[3] | 13.3 | 13.8 | 0.24 | 0.060 |
| Day 12 | 12.6 | 12.8 | 0.31 | 0.719 |
| Wean | 12.7 | 12.7 | 0.32 | 0.916 |
| Prewean mortality, % | 10.5 | 12.4 | 2.24 | 0.557 |

[1]A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from day 30 of gestation until weaning at approximately day 19 of lactation.

[2]Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed and 1,000,000 cfu/g of lactation feed.

[3]Piglets were cross-fostered within sow treatment group to equalize litter size within 24 h of birth.

BW SEM = 0.01) compared to the sow portion of the study (weaning BW SEM = 0.21).

In phase 1, from day 0 to 7 of nursery, there was a tendency ($P$ = 0.088) for interaction of sow dietary treatment and nursery dietary treatment on G:F, where pigs born from control-fed sows had improved G:F when fed the probiotic diet compared to the control diet, but pigs born from probiotic-fed sows had similar G:F when fed the probiotic diet or the control diet. There was no evidence ($P$ > 0.10) for effect of sow dietary treatment on growth performance. There was a tendency ($P$ = 0.084) for effect of nursery dietary treatment on ADG, where pigs fed the probiotic diet in the nursery had increased ADG compared to pigs fed the control diet. However, no evidence ($P$ > 0.10) for effect of nursery dietary treatment was observed on ADFI. Body weight of pigs on day 7 of nursery was not influenced ($P$ > 0.10) by sow or nursery dietary treatments. In phase 2, from day 7 to 21 of nursery, there was no evidence ($P$ > 0.10) for effect of sow or nursery dietary treatments on growth performance. Body weight of pigs on day 21 of nursery was not influenced ($P$ > 0.10) by sow or nursery dietary treatments. In phase 3, from day 21 to 42 of nursery, there was an effect ($P$ < 0.01) of sow dietary treatment on ADG and ADFI, where pigs born from control-fed sows had increased ADG and ADFI compared to pigs born from probiotic-fed sows. However, no evidence ($P$ > 0.10) for effect of sow dietary treatment was observed for G:F. There was a tendency ($P$ = 0.084) for effect of nursery dietary treatment for G:F, where pigs fed the control diet in the nursery had increased G:F compared to pigs fed the probiotic diet. However, no evidence ($P$ > 0.10) for effect of nursery dietary treatment was observed on ADG and ADFI.

Overall, from day 0 to 42 of nursery, there was no evidence ($P$ > 0.10) for effect of sow or nursery dietary treatments on growth performance. There was an effect ($P$ = 0.042) of sow dietary treatment on final nursery BW, where pigs born from control-fed sows were heavier than pigs born from probiotic-fed sows on day 42 of nursery. There was no evidence ($P$ > 0.10) for effect of nursery dietary treatment on final nursery BW.

### *Fecal Score*

Fecal score of nursing and nursery pigs is presented as the frequency distribution of litters and pens, respectively, within each fecal score category. Fecal score of nursing piglets was not influenced ($P$ > 0.10) by interaction of sow dietary treatment by day or main effect of sow dietary treatment (Fig. 1). Fecal consistency was mostly classified as hard feces or firm formed feces in litters from both control- or probiotic-fed sows. There was a tendency

**Table 6.** Interactive effects of sow and nursery dietary treatments on growth performance of nursery pigs[1,2]

| Sow treatment[3] | Control | | Probiotic | | | Probability, $P <$ | | |
|---|---|---|---|---|---|---|---|---|
| Nursery treatment[4] | Control | Probiotic | Control | Probiotic | SEM | Sow treatment × nursery treatment | Sow treatment | Nursery treatment |
| Item | | | | | | | | |
| BW, kg | | | | | | | | |
| Day 0 | 5.8 | 5.8 | 5.9 | 5.9 | 0.01 | 0.995 | 0.001 | 0.547 |
| Day 7 | 6.3 | 6.4 | 6.3 | 6.4 | 0.05 | 0.350 | 0.940 | 0.114 |
| Day 21 | 10.8 | 10.9 | 10.9 | 10.7 | 0.17 | 0.441 | 0.677 | 0.795 |
| Day 42 | 24.0 | 23.9 | 23.3 | 23.1 | 0.34 | 0.841 | 0.042 | 0.707 |
| Phase 1 (day 0 to 7) | | | | | | | | |
| ADG, g | 62 | 82 | 63 | 69 | 7.49 | 0.333 | 0.418 | 0.084 |
| ADFI, g | 113 | 117 | 117 | 119 | 7.42 | 0.853 | 0.704 | 0.681 |
| G:F, g/kg | 506 | 691 | 552 | 550 | 54.35 | 0.088 | 0.383 | 0.093 |
| Phase 2 (day 7 to 21) | | | | | | | | |
| ADG, g | 314 | 317 | 323 | 308 | 10.18 | 0.359 | 0.986 | 0.560 |
| ADFI, g | 435 | 445 | 451 | 427 | 11.85 | 0.151 | 0.959 | 0.549 |
| G:F, g/kg | 720 | 712 | 716 | 722 | 11.49 | 0.562 | 0.764 | 0.905 |
| Phase 3 (day 21 to 42) | | | | | | | | |
| ADG, g | 627 | 612 | 594 | 588 | 9.89 | 0.628 | 0.005 | 0.293 |
| ADFI, g | 931 | 924 | 886 | 880 | 16.46 | 0.980 | 0.008 | 0.702 |
| G:F, g/kg | 674 | 662 | 672 | 669 | 4.24 | 0.293 | 0.679 | 0.084 |
| Overall (day 0 to 42) | | | | | | | | |
| ADG, g | 424 | 422 | 414 | 407 | 8.30 | 0.755 | 0.135 | 0.535 |
| ADFI, g | 623 | 624 | 612 | 600 | 12.32 | 0.595 | 0.146 | 0.661 |
| G:F, g/kg | 681 | 675 | 678 | 678 | 3.83 | 0.491 | 0.998 | 0.478 |

[1]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. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment.

[2]Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[3]Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation).

[4]Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 cfu/g of nursery feed.

($P = 0.070$) for an effect of day in lactation in fecal score of nursing piglets. On day 2 of lactation, fecal consistency was mostly classified as firm formed feces or soft moist feces but, on day 18 of lactation, fecal consistency mostly shifted to hard feces or firm formed feces.

Fecal score of nursery pigs was not influenced ($P > 0.10$) by interactions or main effects of sow dietary treatment and nursery dietary treatment (Fig. 2). Fecal consistency was mostly classified as soft moist feces or soft unformed feces across dietary treatments. There were no interactions of sow dietary treatment and nursery dietary treatment by day in nursery ($P > 0.10$). There was a tendency ($P = 0.077$) for main effect of day in fecal score of nursery pigs (Fig. 3). During the 42-d nursery period, fecal consistency gradually shifted to a looser pattern, with a decrease in frequency distribution of pens with firm formed feces, an increase of pens with soft unformed feces, and presence of pens with watery feces on day 42 of nursery.

### *Fecal Microbial Analysis*

Sow fecal microbial analysis revealed an interaction ($P < 0.01$) between sow dietary treatment and day in lactation on number of *B. subtilis* C-3102 and total *Bacillus* sp. (Table 8). In probiotic-fed sows, the numbers of *B. subtilis* C-3102 and total *Bacillus* sp. increased ($P < 0.05$) from day 30 to 113 of gestation and remained at a constant level in lactation until a day prior to weaning; whereas in control-fed sows, the level of *B. subtilis* C-3102 and total *Bacillus* sp. either decreased or remained at a

**Table 7.** Main effects of sow and nursery dietary treatment on growth performance of nursery pigs[1,2]

| | Sow treatment[3] | | | Probability, | Nursery treatment[4] | | | Probability, |
|---|---|---|---|---|---|---|---|---|
| Item | Control | Probiotic | SEM | *P* < | Control | Probiotic | SEM | *P* < |
| BW, kg | | | | | | | | |
| Day 0 | 5.8 | 5.9 | 0.01 | 0.001 | 5.9 | 5.9 | 0.01 | 0.547 |
| Day 7 | 6.3 | 6.3 | 0.04 | 0.940 | 6.3 | 6.4 | 0.04 | 0.114 |
| Day 21 | 10.8 | 10.8 | 0.12 | 0.677 | 10.8 | 10.8 | 0.12 | 0.795 |
| Day 42 | 23.9 | 23.2 | 0.24 | 0.042 | 23.7 | 23.5 | 0.24 | 0.707 |
| Phase 1 (day 0 to 7) | | | | | | | | |
| ADG, g | 72 | 66 | 5.30 | 0.418 | 62 | 75 | 5.23 | 0.084 |
| ADFI, g | 115 | 118 | 5.25 | 0.704 | 115 | 118 | 5.18 | 0.681 |
| G:F, g/kg | 598 | 551 | 38.43 | 0.383 | 529 | 621 | 37.92 | 0.093 |
| Phase 2 (day 7 to 21) | | | | | | | | |
| ADG, g | 316 | 315 | 7.20 | 0.986 | 318 | 313 | 7.10 | 0.560 |
| ADFI, g | 440 | 439 | 8.38 | 0.959 | 443 | 436 | 8.27 | 0.549 |
| G:F, g/kg | 716 | 719 | 8.13 | 0.764 | 718 | 717 | 8.02 | 0.905 |
| Phase 3 (day 21 to 42) | | | | | | | | |
| ADG, g | 619 | 591 | 7.00 | 0.005 | 610 | 600 | 6.90 | 0.293 |
| ADFI, g | 928 | 883 | 11.64 | 0.008 | 908 | 902 | 11.48 | 0.702 |
| G:F, g/kg | 668 | 670 | 3.00 | 0.679 | 673 | 665 | 2.96 | 0.084 |
| Overall (day 0 to 42) | | | | | | | | |
| ADG, g | 423 | 410 | 5.87 | 0.135 | 419 | 414 | 5.79 | 0.535 |
| ADFI, g | 624 | 606 | 8.71 | 0.146 | 617 | 612 | 8.59 | 0.661 |
| G:F, g/kg | 678 | 678 | 2.71 | 0.998 | 679 | 677 | 2.67 | 0.478 |

[1]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. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment.

[2]Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[3]Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation).

[4]Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 cfu/g of nursery feed.

constant level during gestation and lactation. The numbers of *B. subtilis* C-3102 and total *Bacillus* sp. were increased ($P < 0.05$) in probiotic-fed sows compared to control-fed sows at any stage of gestation and lactation.

The numbers of *Lactobacillus* sp., *C. perfringens*, Enterobacteriaceae, and total anaerobes were influenced ($P < 0.01$) by day in lactation. The number of *Lactobacillus* sp. remained constant during gestation (7.13 and 6.84 $\log_{10}$ cfu/g on days 30 and 113, respectively) but increased in lactation (8.45 $\log_{10}$ cfu/g on day 18; $P < 0.05$). The number of *C. perfringens* decreased during gestation and lactation (8.03, 7.74, and 6.08 $\log_{10}$ cfu/g on day 30 of gestation, day 113 of gestation, and day 18 of lactation, respectively; $P < 0.05$). The number of Enterobacteriaceae remained at a constant level during gestation (7.48 and 7.36 $\log_{10}$ cfu/g on days 30 and 113, respectively) but decreased in lactation (6.57 $\log_{10}$ cfu/g on day 18; $P < 0.05$). The number of total anaerobes slightly reduced during gestation (9.15 and 9.00 $\log_{10}$ cfu/g on days 30 and 113, respectively; $P < 0.05$) but increased in lactation (9.30 $\log_{10}$ cfu/g on day 18; $P < 0.05$). For number of total aerobes, there were no evidence ($P > 0.10$) for interactions or main effects of sow dietary treatment or day in lactation. *Salmonella* spp. was detected on day 113 of gestation in 2 out of 14 fecal samples from control-fed sows (average 5.49 $\log_{10}$ cfu/g) and in 1 out of 15 fecal samples from probiotic-fed sows (4.34 $\log_{10}$ cfu/g), but it was not detectable in fecal samples on day 30 of gestation and day 18 of lactation.

Nursing piglet fecal microbial analysis revealed an interaction ($P < 0.05$) between sow dietary treatment and day in lactation on number of *B. subtilis* C-3102, total *Bacillus* sp., and *Lactobacillus* sp. (Table 9). The number of *B. subtilis* C-3102 increased ($P < 0.05$) from day 2 to 18 of lactation in litters from probiotic-fed sows, whereas remained at a constant level in lactation in litters from control-fed sows. The number of total *Bacillus* sp.

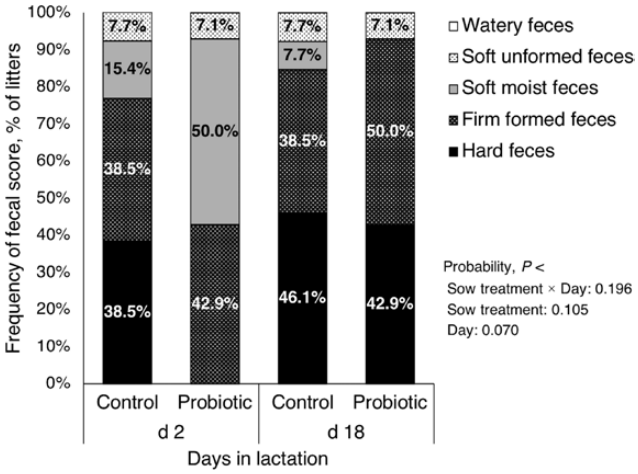


**Figure 1.** Effect of providing *Bacillus subtilis* C-3102 during gestation and lactation on fecal consistency of nursing piglets assessed by fecal score. Graph bars show the frequency distribution of litters ($n$ = 27 litters) within each fecal score category on days 2 and 18 of lactation according to sow dietary treatment. Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation).

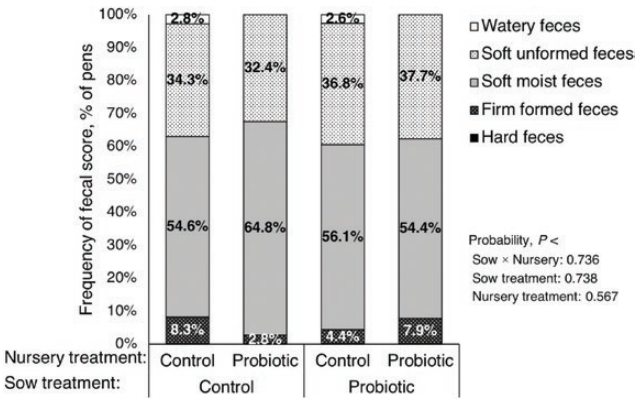


**Figure 2.** Effects of sow and nursery pig dietary treatment on fecal consistency of nursery pigs assessed by fecal score. Graph bars show the frequency distribution of pens ($n$ = 74 pens) within each fecal score category according to sow dietary treatment and nursery dietary treatment regardless of day in nursery. Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation). Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 cfu/g of nursery feed.

decreased ($P$ < 0.05) from day 2 to 18 of lactation in litters from both sow dietary treatments, but the magnitude of decrease was greater in litters from control-fed sows. The numbers of *B. subtilis* C-3102 and total *Bacillus* sp. were increased ($P$ < 0.05) in litters from probiotic-fed sows compared to

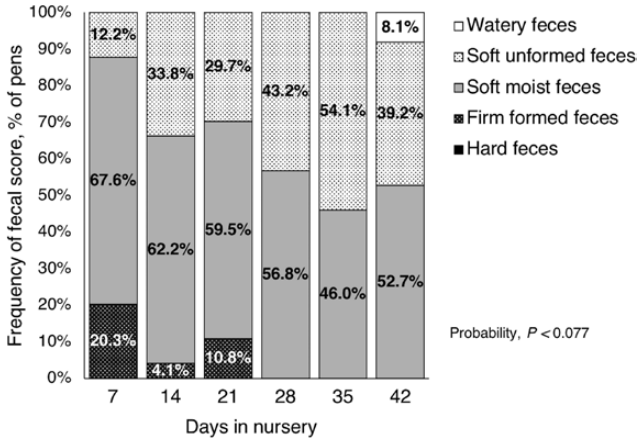


**Figure 3.** Effects of days into the nursery on fecal consistency of nursery pigs assessed by fecal score. Graph bars show the frequency distribution of pens ($n$ = 74 pens) within each fecal score category according to day in nursery regardless of dietary treatment.

control-fed sows on day 18 of lactation. The number of *Lactobacillus* sp. increased ($P$ < 0.05) from day 2 to 18 of lactation in litters from control-fed sows, whereas remained at a constant level in lactation in litters from probiotic-fed sows. The number of *Lactobacillus* sp. was similar in litters from both sow dietary treatments on day 18 of lactation.

The numbers of *C. perfringens*, Enterobacteriaceae, total aerobes, and total anaerobes were influenced ($P$ < 0.10) by day in lactation. The number of *C. 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) decreased ($P$ < 0.10) from day 2 to 18 of lactation. For number of *Enterococcus* sp., there was no evidence ($P$ > 0.10) for interactions or main effects of sow dietary treatment or day in lactation. *Salmonella* spp. was detected on day 2 of lactation in 1 out of 13 fecal samples from litters from control-fed sows (7.33 $\log_{10}$ cfu/g), but it was not detectable in fecal samples on day 18 of lactation.

Nursery pig fecal microbial analysis revealed an interaction ($P$ < 0.01) between sow dietary treatment, nursery dietary treatment, and day in nursery on number of *B. subtilis* C-3102 (Tables 10 and 11). Nursery pigs from control-fed sows and also fed a control diet in the nursery maintained lower levels of *B. subtilis* C-3102 during nursery, whereas pigs from control-fed sows but fed a probiotic diet in the nursery rapidly increased and maintained higher levels of *B. subtilis* C-3102 during the nursery. Nursery pigs from probiotic-fed sows and also fed a probiotic diet in the nursery maintained higher levels of *B. subtilis* C-3102 during the nursery, whereas pigs born from probiotic-fed sows but

**Table 8.** Effects of providing *Bacillus subtilis* C-3102 during gestation and lactation on sow fecal microbes[1, 2]

| | Day 30 gestation | | Day 113 gestation | | Day 18 lactation | | Probability, $P <$ | | |
|---|---|---|---|---|---|---|---|---|---|
| Item[3] | Control | Probiotic[4] | Control | Probiotic[4] | Control | Probiotic[4] | Treatment × day | Treatment | Day |
| *Bacillus subtilis* C-3102, $\log_{10}$ cfu/g | 3.13[c] | 4.69[b] | 1.76[d] | 6.14[a] | 2.69[c] | 6.20[a] | 0.003 | 0.001 | 0.031 |
| SEM | 0.39 | 0.37 | 0.17 | 0.16 | 0.19 | 0.18 | | | |
| Detected/sampled, *n* | 8/10 | 10/10 | 2/14 | 15/15 | 9/14 | 15/15 | | | |
| Total *Bacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 10/10 | 10/10 | 14/14 | 15/15 | 14/14 | 15/15 | | | |
| *Lactobacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 10/10 | 10/10 | 14/14 | 13/15 | 14/14 | 15/15 | | | |
| *Clostridium perfringens*, $\log_{10}$ cfu/g | 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, *n* | 10/10 | 10/10 | 14/14 | 15/15 | 14/14 | 15/15 | | | |
| Enterobacteriaceae, $\log_{10}$ cfu/g | 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, *n* | 10/10 | 10/10 | 14/14 | 15/15 | 14/14 | 15/15 | | | |
| Total aerobes, $\log_{10}$ cfu/g | 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, *n* | 10/10 | 10/10 | 14/14 | 15/15 | 14/14 | 15/15 | | | |
| Total anaerobes, $\log_{10}$ cfu/g | 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, *n* | 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 (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from day 30 of gestation until weaning at approximately day 19 of lactation.

[2]Fecal samples were freshly collected from sows by rectal grab on day 30 of gestation (baseline), day 112 of gestation (prefarrowing), and day 18 of lactation (preweaning). Microbial analysis was performed by isolation and enumeration method.

[3]Limit of detection was $2 \times 10^2$ cfu/g.

[4]Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 lactation).

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

fed a control diet in the nursery gradually decreased and maintained lower levels of *B. subtilis* C-3102 during the nursery. The number of total *Bacillus* sp. was influenced ($P < 0.01$) by nursery dietary treatment, but there was no evidence ($P > 0.10$) for interactions or main effects of sow dietary treatment or day in nursery. Nursery pigs fed a probiotic diet in the nursery had increased number of total *Bacillus* sp. compared to pigs fed a control diet in the nursery (5.69 vs. 4.09 $\log_{10}$ cfu/g, respectively; $P < 0.01$).

The number of total aerobes was influenced ($P < 0.05$) by an interaction between nursery dietary treatment and day in nursery and a main effect of sow dietary treatment. Nursery pigs fed a control diet in the nursery slightly increased the number of total aerobes during nursery (9.52 to 9.70 $\log_{10}$ cfu/g from day 21 to 42; $P < 0.05$), whereas pigs fed the probiotic diet maintained a constant number of total aerobes during nursery (9.58 to 9.57 $\log_{10}$ cfu/g from day 21 to 42; $P > 0.10$). Nursery pigs from control-fed sows had slightly increased number of total aerobes compared to pigs from probiotic-fed sows (9.65 vs. 9.54 $\log_{10}$ cfu/g, respectively; $P < 0.05$).

The number of total anaerobes was influenced ($P < 0.05$) by an interaction between sow dietary treatment and nursery dietary treatment and a main effect of day in nursery. Nursery pigs from control-fed sows and also fed the control diet in the nursery had slightly higher ($P < 0.05$) 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 from probiotic-fed sows (10.10 $\log_{10}$ cfu/g), whereas the number of total anaerobes in pigs born from probiotic-fed sows and also fed the probiotic diet in the nursery was intermediate (10.17 $\log_{10}$ cfu/g). The number of total anaerobes slightly decreased from day 21 to 42 of nursery (10.19 to 10.12 $\log_{10}$ cfu/g; $P < 0.05$).

**Table 9.** Effects of providing *Bacillus subtilis* C-3102 during gestation and lactation on nursing piglet fecal microbes[1,2]

| | Day 2 lactation | | Day 18 lactation | | Probability, $P <$ | | |
|---|---|---|---|---|---|---|---|
| Item[3] | Control | Probiotic[4] | Control | Probiotic[4] | Treatment × day | Treatment | Day |
| *Bacillus subtilis* C-3102, $\log_{10}$ cfu/g | 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, *n* | 5/13 | 9/14 | 7/13 | 14/14 | | | |
| Total *Bacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 11/13 | 14/14 | | | |
| *Lactobacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 12/13 | 14/14 | 13/13 | 14/14 | | | |
| *Enterococcus* sp., $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 13/13 | 14/14 | | | |
| *Clostridium perfringens*, $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 13/13 | 14/14 | | | |
| Enterobacteriaceae, $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 13/13 | 14/14 | | | |
| Total aerobes, $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 13/13 | 14/14 | | | |
| Total anaerobes, $\log_{10}$ cfu/g | 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, *n* | 13/13 | 14/14 | 13/13 | 14/14 | | | |

[1]A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from day 30 of gestation until weaning at approximately day 19 of lactation.

[2]Fecal samples were freshly collected from piglets with sterile mini cotton tip swabs on days 2 (postnatal) and 18 of lactation (preweaning). Microbial analysis was performed by isolation and enumeration method.

[3]Limit of detection was $2 \times 10^2$ cfu/g.

[4]Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 lactation).

[abc] indicate significant difference ($P < 0.05$) in the row.

For *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae, there were only tendencies ($P < 0.10$) for interactions or main effects of sow dietary treatment, nursery dietary treatment, and day in nursery. The practical and biological significance of these tendencies were not considered relevant to the study. *Clostridium perfringens* and *Salmonella* spp. were not detectable in fecal samples in the nursery.

## DISCUSSION

Preweaning piglet development is intrinsically reliant on the sow. The intimate contact of newborn piglets with the sow is an important determinant of early bacterial colonization of the porcine gastrointestinal tract (Everaert et al., 2017) and exerts a long-term influence on pigs described as "microbial imprinting" (Thompson et al., 2008; Mach et al., 2015). Maternal-to-progeny transfer of bacteria originates from the reproductive tract during parturition and from the milk, skin, and fecal–oral contact during lactation (Buddington et al., 2010). However, the balance between beneficial and pathogenic bacteria can be altered during critical periods of the sow reproductive cycle, particularly from farrowing through weaning (Liu et al., 2019). Dietary strategies meant to modulate the bacterial population and re-establish the bacterial balance of sows can confer health benefits to sows and, indirectly, to the progeny (Baker et al., 2013).

Probiotics have been appointed as promising additives to modulate the intestinal microbiota via sow nutrition because, by definition,

**Table 10.** Effects of sow and nursery dietary treatment on nursery pigs fecal microbes[1,2]

| | Day 21 nursery | | | | Day 42 nursery | | | |
|---|---|---|---|---|---|---|---|---|
| Sow treatment[3] | Control | | Probiotic | | Control | | Probiotic | |
| Nursery treatment[4] | Control | Probiotic | Control | Probiotic | Control | Probiotic | Control | Probiotic |
| Item[5] | | | | | | | | |
| *Bacillus subtilis* C-3102, $\log_{10}$ cfu/g | 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, *n* | 4/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 3/6 | 6/6 |
| Total *Bacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |
| *Lactobacillus* sp., $\log_{10}$ cfu/g | 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, *n* | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |
| *Enterococcus* sp., $\log_{10}$ cfu/g | 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, *n* | 6/6 | 5/6 | 6/6 | 5/6 | 6/6 | 5/6 | 6/6 | 6/6 |
| Enterobacteriaceae, $\log_{10}$ cfu/g | 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, *n* | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |
| Total aerobes, $\log_{10}$ cfu/g | 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, *n* | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |
| Total anaerobes, $\log_{10}$ cfu/g | 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, *n* | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |

[1]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. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[2]Fecal samples were freshly collected from piglets with sterile cotton tip swabs on days 21 and 42 of nursery. Microbial analysis was performed by isolation and enumeration method.

[3]Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation).

[4]Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 cfu/g of nursery feed.

[5]Limit of detection was $2 \times 10^2$ cfu/g.

probiotics are nonpathogenic live microorganisms that can improve the intestinal microbial balance and confer health benefits once provided in adequate amounts (Fuller, 1989). Probiotics have been found to influence the developing intestinal microbiota of nursing piglets through supplementation of sows (Baker et al., 2013; Starke et al., 2013). Interestingly, the probiotic influence on intestinal microbiota of the progeny in early life seems to be extended to postweaning stages later in life (Alexopoulos et al., 2001; Silva et al., 2010). In light of the available literature, the present study focused on the further comprehension of the maternal-progeny intestinal microbiota relationship and the long-term impact of providing a probiotic, *B. subtilis* C-3102, to sows on progeny through the nursery in regard to performance, fecal consistency, and fecal microbes.

The findings of the sow portion of the study indicate a benefit of providing *B. subtilis* C-3102 during gestation and lactation on sow lactation feed intake. Previous studies providing *Bacillus* sp. or *Enterococcus* sp. species to sows during late gestation and lactation support an improvement in lactation feed intake with probiotics (Alexopoulos et al., 2004; Böhmer et al., 2006). Feed consumption during lactation is important to achieve the milk production potential to support large and fast-growing litters with minimal mobilization of sow body reserves (Strathe et al., 2017). However, while probiotic-fed sows consumed on average 0.5 kg more feed per day than control-fed sows,

**Table 11.** Probability of interactions and main effects of sow dietary treatment, nursery dietary treatment, and day in nursery on nursery pigs fecal microbes[1,2,3]

| Item | 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 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[2]Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 cfu/g of gestation feed (day 30 gestation to farrowing) and 1,000,000 cfu/g of lactation feed (farrowing to day 19 of lactation).

[3]Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 cfu/g of nursery feed.

the improvement in feed intake did not affect litter performance or sow body weight loss during lactation as previously reported (Alexopoulos et al., 2001, 2004; Stamati et al., 2006; Baker et al., 2013).

The influence of sow probiotic supplementation on litter performance until weaning is not consistent in the literature. While some studies report improvements in weaning weight, number of weaned piglets, fecal consistency, and preweaning mortality driven by sow supplementation with bacillus-based probiotics (Alexopoulos et al., 2001, 2004; Stamati et al., 2006), others including the present study fail to find evidence for improvements in preweaning performance (Böhmer et al., 2006; Baker et al., 2013). Improvements in preweaning performance have been attributed to beneficial effects of probiotics on milk composition and microbial balance (Alexopoulos et al., 2001, 2004; Stamati et al., 2006; Starke et al., 2013), but only the latter has been assessed in the present study. In the present study, only litter size after cross-fostering was improved in probiotic-fed sows by an average of 0.5 piglet per litter compared to control-fed sows, but the litter size advantage was not maintained until weaning. The improvement in litter size after cross-fostering is a consequence of the numerically larger number of piglets born alive in probiotic-fed sows, with an average of 0.4 more piglet born alive per litter compared to control-fed sows. The variation in number of piglets born alive among sows likely limited the ability to find significant differences in litter size at birth, whereas the consistency in litter size after equalization allowed for a significant effect. Nevertheless, it cannot be assumed that litter size at birth is a primary effect of sow probiotic supplementation, particularly starting on day 30 of gestation as in the present study, because litter size is determined in earlier stages of pregnancy and subject to a multitude of unrelated factors (Böhmer et al., 2006; Østrup et al., 2011).

Providing a bacillus-based probiotic to sows during gestation and lactation induced a sow fecal microbial population modification by increasing the number of total *Bacillus* sp. as a consequence of increasing *B. subtilis* C-3102. Most importantly, probiotic supplementation to sows influenced the developing fecal microbial population of the progeny. Piglets born and nursed by probiotic-fed sows displayed a similar fecal microbial population with increasing number of *B. subtilis* C-3102 and total *Bacillus* sp. in the preweaning period. Previous studies support the influence of probiotics on sow fecal microbial population as well as the maternal transfer of probiotic strains to the progeny and the mirrored fecal microbial population in nursing piglets (Taras et al., 2005, 2006). The conditions in the gastrointestinal tract of newborn piglets are permissive for bacterial colonization (Buddington et al., 2010). The modulation of sow fecal microbiota is an effective strategy to reduce pathogen load and establish beneficial bacteria more rapidly in the gastrointestinal tract of piglets in the early postnatal period (Baker et al., 2013).

Studies providing *Bacillus* sp. or *Enterococcus* sp. species to sows during late gestation and lactation describe improvements in the population

of beneficial bacteria, primarily *Lactobacillus* sp., and reductions in the population of potentially harmful bacteria, including *C. perfringens* and *Escherichia coli* (Baker et al., 2013; Starke et al., 2013). However, similar probiotic-driven modifications of fecal microbial population were not found in the present study. The potential of *B. subtilis* C-3102 to increase *Lactobacillus* sp. and decrease Enterobacteriaceae in fecal microbial population of sows and decrease *Clostridium* sp. in the fecal microbial population of the progeny has been demonstrated in previous studies (Maruta et al., 1996b; Kritas et al., 2015). However, the normal microbial population of sows and piglets should be taken into consideration. In the present study, the number of *C. perfringens* in fecal microbial population of sows and piglets was equivalent to or greater than the number of *Lactobacillus* sp. The levels of *C. perfringens* were not causing clinical signs in sows or piglets and were considered within normal levels for the farm under study, as evaluated in other instances before and after the present study. However, it could be speculated that the dose of *B. subtilis* C-3102 used in this study was not enough to displace *C. perfringens* (Maruta et al., 1996b) or to elicit an effect in number of *Lactobacillus* sp. of sufficient magnitude to outnumber *C. perfringens*. This could also explain the lack of probiotic effect on fecal consistency in the preweaning period. Moreover, the intestinal microbiota of sows seems to inherently control the impact of probiotics and, once established and stable, a fundamental change on bacterial population as consequence of probiotic supplementation becomes less likely (Savage et al., 1978).

The findings of the nursery portion of the study indicate a similar growth performance and fecal consistency in the overall nursery period in spite of providing *B. subtilis* C-3102 to sows and/or nursery pigs. Only few studies were designed to evaluate the long-term influence of providing bacillus-based or lactic acid bacteria-based probiotics to sows in late gestation and lactation on the progeny through the nursery (Alexopoulos et al., 2001; Silva et al., 2010). The studies suggest that the beneficial effects of probiotics seem to be additive, as growth rate and weight gain in the nursery are further improved in nursery pigs born from sows fed probiotic diets and also fed probiotic diets in the nursery (Alexopoulos et al., 2001; Silva et al., 2010). In contrast, no additive effects of probiotics were found in the nursery portion of the present study, as implied by the lack of interactions between sow and nursery dietary treatments on nursery performance. The studies also suggest that the effects of probiotics on performance of nursery pigs can be indirect, when pigs are born from sows fed probiotic diets in gestation and lactation, or direct, when pigs are fed probiotic diets in the nursery (Alexopoulos et al., 2001). In the present study, the indirect effect of probiotics in nursery performance was observed in late nursery. Contrarily to expected, nursery pigs born from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery. Although the underlying cause remains unclear, the fact that growth performance impairment only occurred in late nursery suggests that a modification of intestinal microbial population driven by dietary change could be speculated. In the last phase of nursery, pigs were switched to a considerably simpler diet in comparison to the diet composition of previous nursery phases by the removal of lactose sources, specialty protein sources, and pharmacological levels of zinc oxide. Dietary changes have been associated with significant shifts on fecal microbiota of nursery pigs, including structural and functional transitions in the attempt to face a challenge (Tilocca et al., 2017). Although the fecal microbial analysis performed in the present study did not identify differences in fecal microbes between nursery pigs born from control- or probiotic-fed sows, it is plausible to speculate that there could have been more complex differences in microbiota composition not able to be identified by isolation and enumeration of a limited number of bacteria. A difference in basal microbiota composition between nursery pigs born from control- or probiotic-fed sows could have led to distinct microbiota adaptation processes following a change in diet composition and reflected on growth performance (Tilocca et al., 2017). However, the theory presented here warrants further investigations.

The direct effect of probiotics in nursery performance was modest in the present study. In contrast, previous studies with weaned pigs fed diets with *B. subtilis* C-3102 demonstrate the potential to improve growth rate and feed efficiency by 5% to 6% in the nursery with probiotics (Marubashi et al., 2012). However, the inconsistency of growth performance effects to probiotics is commonplace in the probiotic scientific literature (Zimmermann et al., 2016). The variation within the use of the same bacteria strain could be attributed to a multitude of factors, including dietary composition, environmental conditions, and health status. In this regard, it has been suggested that growth-promoting effects of probiotics are more evident under conditions of dietary, environmental, or health challenges

(Madec et al., 1998). In the present study, the health status and sanitation of nursery facilities, as well as the formulation of diets at the nutrient requirements for nursery pigs (NRC, 2012) and the inclusion of pharmacological levels of zinc oxide, might have contributed to the lack of growth-promoting effect of probiotics in diets for nursery pigs. This could also explain the lack of probiotic effect on fecal consistency in the postweaning period.

Providing a bacillus-based probiotic to nursery pigs induced a modest modification in fecal microbial population by increasing the number of total *Bacillus* sp. as a consequence of increasing *B. subtilis* C-3102 irrespective of sow diet in gestation and lactation. Although providing *B. subtilis* C-3102 to sows in gestation and lactation is able to increase total *Bacillus* sp. in fecal microbial population of nursing piglets as discussed previously, the levels of total *Bacillus* sp. are not sustained during nursery without providing probiotic to nursery pigs. This agrees with the characteristic of bacillus-based probiotics, spores of which germinate but not proliferate in the gastrointestinal tract (Buchanan et al., 1974). Moreover, providing *B. subtilis* C-3102 to nursery pigs only seems to elicit the same increase in total *Bacillus* sp. in fecal microbial population as the supplementation of both sows and nursery pigs. However, providing *B. subtilis* C-3102 to both sows and nursery pigs seems to control the number of total aerobes in fecal microbial population of nursery pigs. Total aerobes count is a common indicator of general bacterial population on fecal samples (Buchanan et al., 1974), which indicates that the probiotic contributes to maintaining a low bacterial load in the feces of nursery pigs and, consequently, in the environment (Siggers et al., 2008; Luyckx et al., 2016). However, the number of *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae in fecal microbial population of nursery pigs was not influenced by providing probiotics to sows and/or nursery pigs. Bacillus-based probiotics as the *B subtilis* C-3102 used in the present study are gram-positive, spore-forming bacteria, the main mode of action of which is through the production of enzymes subtilisin and catalase to create a favorable environment for growth and colonization of beneficial bacteria in the gastrointestinal tract, particularly the *Lactobacillus* sp. (Buchanan et al., 1974; Hosoi et al., 2000). However, the probiotic did not elicit an increase in number of *Lactobacillus* sp. in the feces of nursery pigs in the present study, which might have contributed to the lack of probiotic effect on growth performance and fecal consistency of nursery pigs in the present study.

In conclusion, providing a probiotic based on viable spores of *Bacillus subtilis* C-3102 to sows during gestation and lactation and to progeny during nursery did not elicit noteworthy improvements in performance or fecal consistency. The most notable benefit was seen as an improvement of sow lactation feed intake. Interestingly, fecal microbial analysis indicated the establishment of maternal-progeny intestinal microbiota relationship and its modulation by providing the probiotic to sows. In the preweaning period, pigs born from probiotic-fed sows displayed a similar fecal microbial population as sows but no influence on performance. In the postweaning period, however, pigs from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery. Therefore, there seems to be a long-term influence of sow probiotic supplementation on progeny through the nursery that warrants further investigations.

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