ORIGINAL RESEARCH PEER REVIEWED

Effects of oral administration of *Bacillus subtilis* C-3102 to nursing piglets on preweaning growth performance, fecal consistency, and fecal microbes

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Summary

Objective: To evaluate the effects of daily oral dose of *Bacillus subtilis* C-3102 to nursing piglets on fecal consistency, fecal microbes, and preweaning performance in a controlled trial.

Materials and methods: A total of 26 litters of nursing piglets were assigned to receive a daily oral dose of placebo (n = 14 litters) or probiotic (n = 12 litters) for 18 days beginning on day 2 after birth until weaning on day 19. The probiotic treatment was *B subtilis* C-3102 (Calsporin, Calpis Co Ltd). Treatments were applied orally once daily to individual piglets via 1 mL sugar-based gel solution alone (placebo) or with *B subtilis* C-3102. Growth performance and litter size were measured on days 2, 9, 16, and 19. Fecal scoring and sampling were performed on days 2, 9, and 16 to categorize fecal consistency and conduct microbial analysis by isolation and enumeration method.

Results: There was no statistical difference ($P > .05$) on growth performance, litter size, mortality, and fecal consistency in the preweaning period between placebo- and probiotic-treated litters. The numbers of *B subtilis* C-3102 ($P < .001$), total *Bacillus* species ($P < .001$), and total aerobes ($P = .03$) were increased in litters receiving probiotic compared to placebo. The numbers of *Lactobacillus* species, *Enterococcus* species, *Clostridium perfringens*, and Enterobacteriaceae were not influenced by treatment.

Implications: A daily oral dose of *B subtilis* C-3102 probiotic did not influence preweaning growth performance and fecal consistency of nursing piglets and only influenced *Bacillus* species fecal microbial population.

Keywords: swine, *Bacillus subtilis*, diarrhea, fecal bacterial population, suckling pigs

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Resumen – Effectos de la administración oral de *Bacillus subtilis* C-3102 a lechones lactantes sobre el rendimiento del crecimiento previo al destete, la consistencia fecal, y los microbios fecales

Objetivo: Evaluar los efectos de la dosis oral diaria de *Bacillus subtilis* C-3102 en lechones lactantes sobre la consistencia fecal, los microbios fecales y el rendimiento previo al destete en un ensayo controlado.

Materiales y métodos: Se asignó un total de 26 camadas de lechones lactantes para recibir una dosis oral diaria de placebo (n = 14 camadas) o probiótico (n = 12 camadas) durante 18 días a partir del día 2 después del nacimiento hasta el destete el día 19. El tratamiento probiótico fue *B subtilis* C-3102 (Calsporin, Calpis Co Ltd). Los tratamientos, a base de 1 mL de solución de gel solo de azúcar (placebo) o con *B subtilis* C-3102, se aplicaron por vía oral una vez al día individualmente a cada lechón. El crecimiento y el tamaño de la camada se midieron los días 2, 9, 16, y 19. La puntuación fecal y el muestreo se realizaron los días 2, 9, y 16 para clasificar la consistencia fecal y realizar análisis microbianos mediante el método de aislamiento y enumeración.

Resultados: No hubo diferencia estadística ($P > .05$) en el crecimiento, el tamaño de la camada, la mortalidad y la consistencia fecal en el período previo al destete entre las camadas tratadas con placebo y con probióticos. El número de *B subtilis* C-3102 ($P < .001$), el total de especies de *Bacillus* ($P < .001$) y los aerobios totales ($P = .03$) aumentaron en las camadas que recibieron probióticos en comparación con el placebo. El tratamiento no influyó en el número de especies de *Lactobacillus*, *Enterococcus*, *Clostridium perfringens* y Enterobacteriaceae.

Implicaciones: Una dosis oral diaria de probiótico *B subtilis* C-3102 no influyó en el rendimiento del crecimiento previo al destete y ni en la consistencia fecal de los lechones lactantes y solo influyó en la población microbiana fecal de las especies de *Bacillus*.

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Résumé – Effets de l'administration de *Bacillus subtilis* C-3102 à des porcelets en pouponnière sur les performances de croissance pré-sevrage, la consistance des fèces, et les microbes fécaux

Objectif: Évaluer les effets d'une dose orale quotidienne de *Bacillus subtilis* C-3102 à des porcelets en pouponnière sur la consistance des fèces, les microbes fécaux, et les performances pré-sevrage dans un essai contrôlé.

Matériels et méthodes: Vingt-six portées de porcelets en pouponnière furent assignées à recevoir une dose orale quotidienne de placebo (n = 14 portées) ou un probiotique (n = 12 portées) pendant 18 jours débutant au jour 2 suivant la mise-bas jusqu'au sevrage au jour 19. Le traitement probiotique était *B subtilis* C-3102 (Calsporin, Calpis Co Ltd). Les traitements furent appliqués oralement une fois par jour individuellement aux porcelets via 1 mL d'une solution en gel à base de sucre seulement (placebo) ou avec *B subtilis* C-3102. Les performances de croissance et la taille de la portée furent mesurées aux jours 2, 9, 16, et 19. Un pointage des fèces et des échantillonnages furent effectués aux jours 2, 9, et 16 afin de caractériser la consistance des fèces et mener des analyses microbiologiques par des méthodes d'isolement et de dénombrement.

Résultats: Il n'y avait pas de différence statistiquement significative ($P > .05$) dans les performances de croissance, la taille des litières, les mortalités, et la consistance fécale durant la période pré-sevrage entre les portées ayant reçu le placebo ou celles recevant le probiotique. Le nombre de *B subtilis* C-3102 ($P < .001$), le total d'espèces de *Bacillus* ($P < .001$), et le nombre total de bactéries aérobies ($P = .03$) étaient augmentés chez les portées recevant le probiotique comparativement à celles recevant le placebo. Le nombre d'espèces de *Lactobacillus* et d'*Enterococcus*, le nombre de *Clostridium perfringens*, et d'Enterobacteriaceae n'était pas influencé par le traitement.

Implications: Une dose orale quotidienne de *B subtilis* C-3102 probiotique n'a pas influencé les performances de croissance pré-sevrage et la consistance des fèces de porcelets en pouponnière et influença uniquement les populations microbiennes fécales des espèces de *Bacillus*.

Strategies to improve pig performance and preserve health while minimizing the use of antibiotics are of great interest for the swine industry. The preweaning period is particularly important to focus efforts on improving piglet viability and survivability as preweaning mortality rate typically ranges between 10% to 20% in commercial swine production.[1] Moreover, diarrhea incidence in nursing piglets contributes to poor growth rate and low survivability before weaning as well as a rise in antibiotic use.[2,3]

Porcine gastrointestinal tract bacterial colonization begins at birth and influences the gastrointestinal tract structural, functional, and immunological maturation in neonatal piglets.[4,5] Studies suggest establishing a healthy intestinal microbiota in early life might be essential for preventing pathogen colonization and immune system stimulation later in life.[6-9] Dietary strategies meant to modulate piglet intestinal microbiota during the preweaning period can ultimately lead to these expected health benefits.

Probiotics are non-pathogenic live microorganisms that provided in adequate amounts can improve the intestinal microbial balance and confer a health benefit to the host.[10] *Bacillus subtilis* C-3102 is a nongenetically modified strain of a gram-positive spore-forming bacteria used as a probiotic for swine. The effects of *B subtilis* C-3102 on fecal microbiota have been associated with increase of beneficial bacteria population in sows, particularly *Lactobacillus* species, and reduction of pathogenic bacteria population and diarrhea incidence in the nursing progeny.[11,12] However, to the best of the authors' knowledge, the investigation of direct administration of this bacillary probiotic to nursing piglets has not previously been conducted.

The objective of this study was to evaluate the effects of a daily oral dose of a bacillary probiotic administered to piglets during the nursing phase on fecal consistency, fecal microbes, and preweaning performance.

Materials and methods

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment.

Facilities and health status

The experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, Kansas during a 20-day period in December. The facility was a farrow-to-finish operation with approximately 120 sows in a 5-week batch farrowing system. Replacement gilts were routinely introduced into the herd from the genetic supplier (DNA Genetics) after a quarantine period. Sows were individually housed in environmentally controlled and mechanically ventilated gestation and farrowing barns. All sows were housed within a single gestation barn and a single farrowing room.

The herd was free of porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus. Sows were routinely vaccinated on every reproductive cycle for parvovirus, leptospirosis, and erysipelas (FarrowSure Gold, Zoetis), for enterotoxigenic *Escherichia coli* and *Clostridium perfringens* type C (LitterGuard LT-C, Zoetis), and with a bacterin for *Haemophilus parasuis*. Piglets were vaccinated for porcine circovirus type 2 and *Mycoplasma hyopneumoniae* (Circumvent PCV-M G2, Merck Animal Health) at 1 and 8 weeks of age, and *Lawsonia intracellularis* (Porcilis Ileitis, Merck Animal Health) at 1 week of age. Sows and piglets were administered intramuscular antimicrobial treatment following veterinary directions in the occurrence of clinical signs of bacterial disease.

Animals, housing, and management

A total of 26 lactating sows (DNA 241, DNA Genetics; 2.5 average parity) and litters (412 piglets DNA 241 × 600, DNA Genetics) were used in the study. Initially, a total of 28 sows and litters were allocated to the experiment, consisting of the maximum number of animals in the batch available at the time of experiment. Two sows were removed before the beginning of the study due to postpartum dysgalactia syndrome. Sows were individually housed in an environmentally controlled and mechanically ventilated farrowing house from day 110 of gestation to weaning on day 19 of lactation. Farrowing stalls were equipped with an individual water nipple and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies). Sows were fed 2.7 kg of feed per day until farrowing and gradually transitioned to *ad libitum* feed intake after parturition. Farrowing stalls were equipped with a rubber mat and heating lamp for piglet comfort. Piglets had free access to sow milk and water and no creep feed was provided during lactation. Piglets were processed and cross-fostered to equalize litter size within 24 hours of birth.

Treatments

Treatments were assigned to litters of nursing piglets in a randomized complete block design based on sow parity and farrowing date. Within a farrowing date, sows were blocked by parity and litters were randomly assigned to 1 of 2 treatments using a

spreadsheet-based randomization procedure. Treatments consisted of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets for a period of 18 days beginning on day 2 after birth until weaning on day 19 of lactation. The probiotic treatment was a probiotic product containing *B subtilis* C-3102 (Calsporin, Calpis Co Ltd) provided at approximately 20×10^6 colony-forming units (CFU) per kg of body weight (BW). A daily dosage of 45.0×10^6, 77.5×10^6, and 108.3×10^6 CFU/mL was used on days 2 to 8, 9 to 15, and 16 to 19, respectively. Treatments were applied orally to individual piglets using a dosing device once daily at approximately 7AM via 1 mL gel solution. The gel solution was composed of a sugar-based carrier (Headstart, Animal Science Products, Inc) administered alone or with *B subtilis* C-3102 for placebo or probiotic treatments, respectively. The preparation of the solution consisted of dissolving the carrier in warm water with or without *B subtilis* C-3102 while continuously mixing the solution with a magnetic stirrer. The solution was prepared immediately before use. Both placebo and probiotic suspensions were analyzed for quantification of *B subtilis* C-3102.

Growth performance

Piglets were individually weighed, and litter size recorded on days 2, 9, 16, and 19 (weaning day). Piglet average daily gain (ADG) was calculated from piglet BW gain during each period: days 2 to 8, 9 to 15, 16 to 19, and 2 to 19. Preweaning mortality was calculated from litter size on days 2 and 19. Sow farrowing performance was recorded as number of piglets born, born alive, stillborn, and mummified. Sows were weighed on days 2 and 19 to calculate lactation BW loss. Sow feed intake was recorded daily from days 2 to 19 to calculate overall average lactation feed intake.

Fecal score

Fecal scoring was conducted on days 2, 9, and 16 to categorize the consistency of piglets' feces per litter into the following categories: hard feces, firm formed feces, soft moist feces, soft unformed feces, and watery feces. Fecal score evaluation was conducted by a trained individual blind to treatments.

Fecal microbial analysis

Fecal samples were collected from piglets on days 2, 9, and 16 for microbial analysis. Fecal samples were freshly collected from piglets using sterile mini cotton tip swabs and pooled by litter for analysis. Fecal samples were kept at 4°C until analysis within 24 hours of collection.

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

For microbial plating, 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.[11] Aliquots of 0.05 mL of each dilution were inoculated into selective and non-selective 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 day.[13] Total *Bacillus* species were enumerated by chromogenic method using a differential medium (92325 *Bacillus* ChromoSelect Agar, Sigma-Aldrich) after incubation for 1 day and spores were quantified after incubation at 80°C for 15 minutes.[12] *Lactobacillus* species were enumerated on modified lactobacilli selective agar after anaerobic incubation for 2 days.[11] *Enterococcus* species were enumerated on triphenyltetrazolium chloride-acridine orange-thallous sulfate aesculin crystal violet agar after incubation for 2 days.[11] *Clostridium perfringens* were enumerated on neomycin-brilliant green-taurocholate-nagler agar after anaerobic incubation for 3 days.[11] *Salmonella* species were enumerated on mannitol lysine crystal violet brilliant green agar after incubation for 1 day.[14] Enterobacteriaceae were enumerated on neomycin-brilliant green-taurocholate-blood agar after incubation for 1 day.[11] Total aerobes were enumerated on trypticase soy agar after incubation for 2 days.[11] Total anaerobes were enumerated on glucose blood liver agar and Eggerth-Gagnon agar after anaerobe incubation for 3 days.[11] Limit of detection was 2×10^2 CFU/g. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc.

Statistical analysis

The experiment was a randomized complete block design with sow parity within farrowing date serving as the block and litter as the experimental unit. A total of 13 blocks were used with no replicates within block. Data were analyzed using a linear mixed model with treatment included as fixed effect and block as random effect.

Model assumptions were met by evaluating studentized residuals and QQ plots. All response variables were analyzed assuming a normal distribution unless otherwise noted. Preweaning mortality was analyzed assuming a binomial distribution and fecal score assuming a multinomial distribution. For binomial responses, the logit link function was used and for fecal score the cumulative probit link function was used. Fecal score and fecal microbial analysis were analyzed as repeated measures. Piglet initial BW (day 2) was included as a covariate for piglet BW and ADG during lactation. Statistical models were fit and pairwise comparisons were performed using the GLIMMIX procedure of SAS (SAS Institute Inc). Results were considered significant at $P < .05$.

Results

Quantification of *Bacillus subtilis* C-3102

Quantification of *B subtilis* C-3102 in the oral suspension provided daily to piglets revealed undetectable levels in the placebo, and 7.9×10^8, 10.4×10^8, and 9.8×10^8 CFU/mL in the probiotic treatment for days 2 to 8, 9 to 15, and 16 to 19, respectively.

Performance

Analysis of sow performance demonstrated no statistical difference on farrowing and lactation performance between treatments (Table 1). For nursing piglet performance, no statistical difference was observed in the preweaning period between treatments (Table 2).

Fecal score

Fecal score of nursing piglets was not influenced by treatment ($P = .92$) or treatment by day ($P = .30$) interaction, as observed by the similar frequency distribution of fecal score categories on both placebo- and probiotic-treated litters within lactation day (Figure 1). Fecal score of nursing piglets was influenced ($P < .001$) by day of lactation, as observed by the shift in frequency distribution of fecal score categories throughout the lactation period regardless of treatment (Figure 1). The frequency of firm formed and hard feces increased from day 2 to 9 of lactation, suggesting hardening of feces in the first week of study. Then, from day 9 to 16, the frequency

Table 1: Analysis of sow performance according to litter treatment*

	Placebo	Probiotic	SEM	*P*†
Parity	2.6	2.5	0.23	.30
Total born, No.	17.7	17.5	0.90	.85
Born alive, No.	16.5	16.3	0.64	.80
Stillborn, No.	0.6	0.8	0.26	.59
Mummified, No.	0.6	0.4	0.25	.33
Lactation feed intake, kg	6.60	6.64	0.182	.57
Lactation body weight loss, kg	6.25	6.24	2.659	.99

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co Ltd).
† Level of significance is $P < .05$ using linear mixed models.
SEM = standard error of the mean.

Table 2: Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on preweaning piglet performance*

	Placebo	Probiotic	SEM	*P*†
Body weight, kg				
d 2‡	1.63	1.53	0.042	.07
d 9	2.95	3.04	0.054	.30
d 16	4.76	4.81	0.107	.78
d 19	5.47	5.55	0.136	.67
ADG, g				
d 2 to 8	196	208	7.75	.30
d 9 to 15	259	252	9.51	.63
d 16 to 19	226	247	21.38	.40
d 2 to 19	205	209	7.15	.67
Litter size, No.				
d 2	16.0	15.7	0.23	.31
d 9	15.7	15.1	0.23	.07
d 16	14.9	14.8	0.23	.80
d 19	14.8	14.7	0.26	.92
Mortality, %				
d 2 to 19	7.5	5.8	0.02	.51

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co Ltd).
† Level of significance is $P < .05$ using linear mixed models.
‡ Piglet initial body weight included as a covariate for piglet body weight and ADG during lactation in the statistical analysis.
SEM = standard error of the mean; ADG = average daily gain.

of soft moist and soft unformed feces increased, suggesting a shift to a looser fecal consistency in the second week of study.

Fecal microbial analysis

Fecal microbial analysis revealed an interaction between treatment and day of lactation on number of *B subtilis* C-3102 ($P < .001$), total *Bacillus* species ($P < .001$), and total anaerobes ($P = .03$; Table 3). The numbers of *B subtilis* C-3102 and total *Bacillus* species increased ($P < .001$) in litters receiving probiotic compared to placebo on days 9 and 16 of lactation. On day 2 of lactation, the detection of *B subtilis* C-3102 also increased ($P = .02$) in probiotic litters compared to placebo litters, but total *Bacillus* species was similar ($P = .17$) between litter treatments. The levels of *B subtilis* C-3102 and total *Bacillus* species in placebo litters gradually increased throughout lactation, whereas the levels in probiotic litters considerably increased from day 2 to 9 and then remained constant until day 16 (Table 3). The presence of *B subtilis* C-3102 in fecal microflora of placebo litters is associated to the ubiquitous nature of the species and is within expectations, ie, at least 1 $\log_{10}$ CFU/g lower than fecal microflora of probiotic litters.[13] The quantification of *B subtilis* C-3102 in the placebo oral suspension was undetectable.

The levels of total anaerobes in placebo litters remained constant ($P = .31$) from day 2 to 9 and then decreased ($P < .001$) until day 16, whereas, the levels in probiotic litters increased ($P = .05$) from day 2 to 9 and then decreased ($P < .001$) until day 16. The number of total aerobes was influenced by treatment ($P = .03$) and day of lactation ($P < .001$). The number of total aerobes was increased ($P = .03$) in placebo litters compared to probiotic litters (8.79 vs 8.64 $\log_{10}$ CFU/g, respectively; standard error of the mean [SEM] = 0.046) and the levels decreased ($P < .001$) throughout lactation irrespective of treatment (9.30, 8.53, and 8.32 $\log_{10}$ CFU/g on days 2, 9, and 16, respectively; SEM = 0.066).

The number of *Lactobacillus* species, *Enterococcus* species, and Enterobacteriaceae were influenced ($P < .001$) by day of lactation (Table 3). The number of *Lactobacillus* species increased from day 2 to 9 and then decreased until day 16 of lactation (7.94, 8.85, and 8.47 CFU/g, respectively; SEM = 0.074; $P < .001$). The number of *Enterococcus* species (8.66, 7.42, and 6.06 CFU/g on days 2, 9, and 16, respectively; SEM = 0.151) and Enterobacteriaceae (9.13, 8.33, and 7.36 CFU/g on days 2, 9, and 16, respectively; SEM = 0.074; $P < .001$) decreased throughout lactation.

The number of *C perfringens* was not influenced ($P = .33$) by litter treatment and remained constant ($P = .66$) throughout lactation (Table 3). The fecal microbial analysis revealed non-detectable levels of *Salmonella* species in piglets' feces with exception of one placebo litter sample on day 2 of lactation with 2.75×10^7 CFU/g.

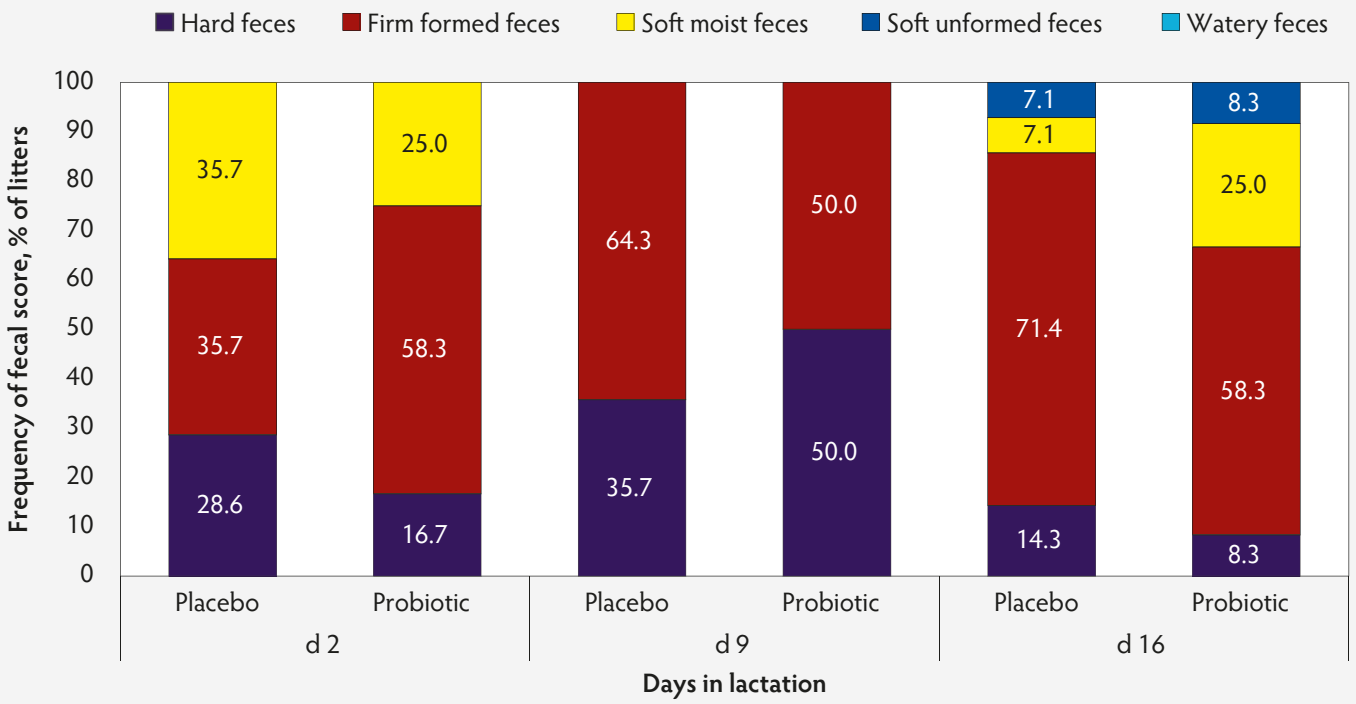
Discussion

Bacterial colonization of the porcine gastrointestinal tract begins at birth and mainly comes from the sow and the environment surrounding the newborn piglet. The first 2 weeks of life have been reported as a developmental window for piglets,[6] in which the gastrointestinal tract is undergoing critically important steps of development including structural, functional, and immunological maturation concomitantly with the establishment of the gut microbiota.[4,5] The establishment of the gut microbiota in early stages of life exerts a long-term influence on pigs described as microbial imprinting,[15] particularly in terms of pathogen colonization and immune system development on the adult pig.[6-9] The evidence that gut microbiota is critically determined at early stages of life presents an opportunity to develop dietary strategies to modulate the gut microbiota of piglets and ultimately lead to an impact on lifetime performance. Because it is difficult to induce a change once the gut microbiota is established and stable,[16] early after birth represents the best opportunity to modulate gut microbiota with dietary strategies.[17] The delivery of probiotics has been recently appointed as a promising additive to piglet nutrition as studies have shown a beneficial impact on growth performance and health of nursing piglets orally supplemented with probiotics in the preweaning period.[18-21] However, to the best of the authors' knowledge, this is the first published study with bacillary probiotics directly administered to nursing piglets.

The delivery of nutritional strategies to nursing piglets is often challenging, even for research purposes. Different strategies have been proposed for early administration of probiotics to piglets, including via sow milk, creep feeding, or suspension in water or milk replacers. The administration of probiotics via sow milk provides dual benefits to sows and piglets, as probiotics are fed to sows and are able to modulate milk bacterial population through the entero-mammary pathway.[22] However, the origin of milk bacterial population is complex and influenced by the bacterial population on the sow skin and in the environment.[23] Moreover, from a research standpoint, it is difficult to determine a standard amount of probiotic being delivered by the milk and consumed by the piglets during lactation. The traditional approach to nutritional supplementation of nursing piglets is via creep feeding. However, studies have shown that not all piglets consume creep feed and those that consume have low intake during the nursing period.[24] Again, from a research standpoint, it is difficult to determine a standard amount of probiotic being consumed by the piglets in the creep feed during lactation. A new approach undertaken by recent studies on probiotic supplementation of nursing piglets consists of individual oral administration of the probiotic in liquid or gel suspension.[18-20] The approach is labor intensive for regular farm application, but practical for research purposes. Most importantly, the direct oral administration to individual piglets ensures the delivery of an accurate dose of probiotics to every piglet in a litter. The consistent delivery of probiotics to nursing piglets was the main reason for choosing the oral administration approach in the present study.

Sow performance at farrowing and during lactation was similar for placebo- and probiotic-treated litters which was expected and thereby not likely to influence the litter response to treatments. The nursing piglet performance in the preweaning period was not influenced by providing a daily oral dose of probiotic until weaning. In contrast, previous studies evaluating the effects of oral administration of probiotic to nursing piglets have found a growth rate improvement ranging from 7% to 15% in litters supplemented with probiotics from the first days after birth until 5 to 21 days of age.[18-21] The fecal consistency of nursing piglets was also not influenced by probiotic administration. The preweaning fecal consistency was mostly classified as firm formed feces and the frequency distribution of fecal score categories was similar in placebo- and probiotic-treated litters during the nursing period. In contrast to our study, a reduction in diarrhea incidence and severity along with improvement in growth

Figure 1: Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on frequency distribution of fecal consistency assessed by litter fecal score. A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co Ltd). Fecal score evaluation was conducted by a trained individual blind to treatments to categorize the consistency of piglets' feces per litter. Interactive and main effects of treatment and day evaluated using linear mixed models.



performance has been observed in previous studies where nursing piglets received early administration of probiotics.[18-21]

The divergence between our study and the literature could be related to the use of different probiotic bacteria with distinct modes of action. In previous studies,[18-21] nursing piglets received lactic acid bacteria-based probiotics, including species of *Lactobacillus* and *Enterococcus*, whereas in the present study piglets received a *Bacillus*-based probiotic. Lactic acid bacteria are gram-positive, non-sporulating bacteria that produce lactic acid as the main metabolic product of carbohydrate fermentation.[25] The lactic acid produced by bacteria contributes to an acidic environment in the gastrointestinal tract to a level which influences growth of pathogenic bacteria. In addition, lactic acid bacteria colonize the intestine and inhibit pathogenic bacteria by competitive exclusion for nutrients or binding sites on the intestinal epithelium.[26] Consequently, the reduction in pathogen load can contribute to an improvement in piglet growth rate.[19]

Bacillus-based probiotics such as the *B subtilis* C-3102 used in the present study are gram-positive, spore-forming bacteria that germinate but not proliferate in the gastrointestinal tract.[25] The germination of *B subtilis* spores results in blocking pathogenic bacteria binding sites on the intestinal epithelium. However, the main mode of action of *Bacillus*-based probiotics is through the production of enzymes subtilisin and catalase as metabolites.[27] The enzymes create a favorable environment for growth and colonization of beneficial bacteria in the gastrointestinal tract, particularly *Lactobacillus* species. However, in the present study the administration of *B subtilis* C-3102 to nursing piglets did not elicit an increase in number of *Lactobacillus* species in the feces. This could explain the lack of probiotic effect on preweaning growth performance and fecal consistency of nursing piglets in the present study. Importantly, the normal microbial population of the piglets should be taken into consideration. In the present study, the number of *Lactobacillus* species in fecal microbial population of nursing piglets was almost equivalent to the number of *C perfringens*. The high levels of *C perfringens* were not causing diarrhea in piglets and were considered within normal levels for the farm under study, as evaluated in other instances before and after the present study. It could be speculated that the dose of *B subtilis* C-3102 used in this study was not enough to influence the high fecal levels of *C perfringens*[11] or to elicit an effect in the number of *Lactobacillus* species so as to outnumber *C perfringens*.

The fecal microbial population of nursing piglets was moderately influenced by providing a daily oral dose of probiotic until weaning. The number of total *Bacillus* species increased in the fecal microbial population of piglets from probiotic-treated litters compared to piglets from placebo-treated litters. The increase in total *Bacillus* species was mainly driven by *B subtilis* C-3102, which was expected to be found in increased number in fecal microbial population of litters receiving the probiotic. The presence of substantial levels of *B subtilis* C-3102 in fecal microbial population of probiotic-treated litters also substantiates

Table 3: Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on fecal microbes*

	Placebo			Probiotic			*P*†		
Microbe, log_{10} CFU/g	**d 2**	**d 9**	**d 16**	**d 2**	**d 9**	**d 16**	**Day**	**Treatment**	**Treatment × Day**
Bacillus subtilis C-3102	2.02^{bx}	2.36^{by}	3.20^{bz}	2.24^{ax}	5.55^{ay}	5.74^{ay}	< .001	< .001	< .001
SEM	0.06	0.10	0.08	0.06	0.11	0.08			
Detected/sampled, No.	2/14	7/14	14/14	7/12	12/12	12/12			
Total *Bacillus* species	2.44^{x}	3.32^{by}	3.75^{bz}	2.67^{x}	5.55^{ay}	5.75^{ay}	< .001	< .001	< .001
SEM	0.13	0.10	0.12	0.13	0.11	0.12			
Detected/sampled, No.	10/14	14/14	14/14	11/12	12/12	12/12			
Lactobacillus species	7.84	8.85	8.48	8.04	8.84	8.45	< .001	.62	.72
SEM	0.16	0.06	0.10	0.19	0.06	0.11			
Detected/sampled, No.	14/14	14/14	14/14	11/11	12/12	12/12			
Enterococcus species	8.58	7.59	5.41	8.74	7.25	6.70	< .001	.18	.10
SEM	0.11	0.19	0.52	0.11	0.21	0.56			
Detected/sampled, No.	13/13	14/14	12/14	10/10	12/12	12/12			
Clostridium perfringens	8.74	8.79	8.59	8.72	8.84	8.89	.66	.33	.40
SEM	0.02	0.13	0.15	0.02	0.14	0.17			
Detected/sampled, No.	14/14	14/14	14/14	12/12	12/12	12/12			
Enterobacteriaceae	9.20	8.33	6.97	9.05	8.34	7.75	< .001	.16	.13
SEM	0.10	0.09	0.27	0.11	0.10	0.29			
Detected/sampled, No.	14/14	14/14	14/14	12/12	12/12	11/12			
Total aerobes	9.32	8.64	8.41	9.28	8.42	8.24	< .001	.03	.66
SEM	0.09	0.09	0.09	0.10	0.10	0.10			
Detected/sampled, No.	14/14	14/14	14/14	12/12	12/12	12/12			
Total anaerobes	9.68^{x}	9.61^{x}	9.27^{y}	9.61^{y}	9.76^{x}	9.18^{z}	< .001	.99	.03
SEM	0.08	0.06	0.07	0.08	0.07	0.08			
Detected/sampled, No.	14/14	14/14	14/14	12/12	12/12	12/12			

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co Ltd). Microbial analysis of fecal samples was performed by isolation and enumeration method.

† Interactive and main effects of treatment and day. Level of significance is $P < .05$ using linear mixed models.

[a,b] Indicate significant difference ($P < .05$) between treatments within each day.

[x,y,z] Indicate significant difference ($P < .05$) between days within each treatment.

CFU = colony-forming units; SEM = standard error of the mean.

our decision to orally dose piglets individually in this study as a means of ensuring the ingestion of the expected dose of probiotic by all piglets in the litters assigned to the probiotic treatment. The number of total aerobes was decreased in fecal microbial population of piglets receiving probiotic compared to piglets receiving placebo. Total aerobe count is commonly used as an indicator of general bacterial population in fecal samples.[25] The decrease in number of total aerobes indicates the probiotic contributes to maintaining a low bacterial load in the feces of nursing piglets and, consequently, in the environment.[28,29] The number of total anaerobes was mostly similar in placebo- or probiotic-treated litters, with both achieving a decrease in number of total anaerobes at the end of lactation. Total anaerobe count is commonly used as an indicator of anaerobic populations in the posterior portion of the gastrointestinal tract, which includes *Lactobacillus*, *Bacteroides*, and *Streptococcus* species among others.[25] In the present study, approximately 90% of the total anaerobes in both placebo- or probiotic-treated litters consisted primarily of *Lactobacillus* species, which is in agreement with previous studies with young piglets.[30]

The number of *Lactobacillus* species, *Enterococcus* species, *C perfringens*, and Enterobacteriaceae in fecal microbial populations was not influenced by providing probiotics to nursing piglets. However, earlier studies have indicated the potential to increase *Lactobacillus* species and decrease Enterobacteriaceae in the fecal microbial population of sows in a before-and-after study with *B subtilis* C-3102.[11]

Recently, a study demonstrated a decrease in *Clostridium* species in the fecal microflora of one-week-old progeny of sows fed *B subtilis* C-3102 probiotic following two sequential reproductive cycles.[12] The lack of influence of *B subtilis* C-3102 on fecal populations of *Lactobacillus* species, *Enterococcus* species, *C perfringens*, and Enterobacteriaceae in nursing piglets in the present study could be due to the same hypothesized reason for the lack of effect on growth performance and fecal consistency: the dose of *B subtilis* C-3102 was not enough to influence the fecal levels of *Enterococcus* species, *C perfringens*, and Enterobacteriaceae or to elicit an increase in *Lactobacillus* species. Furthermore, the fecal population of these bacteria remaining unaffected by the probiotic treatment could be responsible for the lack of effect on preweaning growth performance and fecal consistency of nursing piglets during lactation. Finally, a variation in probiotic effect could be attributed to a multitude of factors, including environmental conditions and health status. In this regard, it has been suggested that growth-promoting effects of probiotics are more evident under conditions of environmental stress or health challenge,[31] which were not experienced in the current study. The effects of *B subtilis* C-3102 probiotic on preweaning performance should be evaluated under typical environmental stress and health challenges of commercial swine production in further studies.

Implications

Under the conditions of this study, providing a daily oral dose of *Bacillus subtilis* C-3102 probiotic to nursing piglets until weaning:

- Did not influence preweaning growth performance and fecal consistency.
- Influenced only total *Bacillus* species fecal microbial populations.

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Conflict of interest

None reported.

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

°F = (°C × 9/5) + 32
°C = (°F - 32) × 5/9

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg
1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne
1 ppm = 1 mg/L