Effect of Calsporin on Nursing Piglet Growth Performance and Fecal Microflora

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Abstract
A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a discovery study to evaluate the effects of Calsporin, a direct-fed microbial containing Bacillus subtilis C-3102, on fecal microflora of nursing pigs. The treatments consisted of providing a daily oral dose of Calsporin or a placebo control to piglets during the nursing phase. Sows were randomly assigned to treatments based on farrowing date, parity, and initial BW. The treatments were applied individually to piglets once a day from d 2 after farrowing and equalization until weaning on d 19. Sow BW, sow ADFI, piglet BW, piglet weight gain, litter size, and mortality were recorded on a weekly basis until weaning. Fecal scoring was conducted to categorize the consistency of the feces using a numerical scale from 1 to 5. Also, fecal samples were collected directly from the rectum of the piglets and pooled by litter for microbial analysis. Fecal scoring and microbial analysis were performed on d 2 after birth, and after 1 or 2 weeks of treatment. As expected, and not a primary objective of this study, there was no evidence for differences among treatments on sow and litter performance (P > 0.085). There was no evidence for differences on fecal score at the beginning of the trial and after 1 or 2 weeks of Calsporin supplementation (P > 0.358). Microbial analysis revealed an increase in levels of total Bacillus sp. (P < 0.001) and a decrease in total aerobes (P < 0.026) in litters treated with Calsporin. There was no evidence for differences in number of Lactobacillus sp., Enterococcus sp., Clostridium perfringens, Enterobacteriaceae, and total anaerobes between control- and Calsporin-treated litters (P > 0.05). In conclusion, once per day supplementation of Calsporin to nursing pigs resulted in slight changes in fecal microflora, but there was no influence on nursing pig fecal consistency.

Keywords
Bacillus subtilis, diarrhea, lactation, probiotic

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

Authors
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Summary
A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a discovery study to evaluate the effects of Calsporin, a direct-fed microbial containing Bacillus subtilis C-3102, on fecal microflora of nursing pigs. The treatments consisted of providing a daily oral dose of Calsporin or a placebo control to piglets during the nursing phase. Sows were randomly assigned to treatments based on farrowing date, parity, and initial BW. The treatments were applied individually to piglets once a day from d 2 after farrowing and equalization until weaning on d 19. Sow BW, sow ADFI, piglet BW, piglet weight gain, litter size, and mortality were recorded on a weekly basis until weaning. Fecal scoring was conducted to categorize the consistency of the feces using a numerical scale from 1 to 5. Also, fecal samples were collected directly from the rectum of the piglets and pooled by litter for microbial analysis. Fecal scoring and microbial analysis were performed on d 2 after birth, and after 1 or 2 weeks of treatment. As expected, and not a primary objective of this study, there was no evidence for differences among treatments on sow and litter performance (P > 0.085). There was no evidence for differences on fecal score at the beginning of the trial and after 1 or 2 weeks of Calsporin supplementation (P > 0.358). Microbial analysis revealed an increase in levels of total Bacillus sp. (P < 0.001) and a decrease in total aerobes (P < 0.026) in litters treated with Calsporin. There was no evidence for differences in number of Lactobacillus sp., Enterococcus sp., Clostridium perfringens, Enterobacteriaceae, and total anaerobes between control- and Calsporin-treated litters (P > 0.05). In conclusion, once per day supplementation of Calsporin to nursing pigs resulted in slight changes in fecal microflora, but there was no influence on nursing pig fecal consistency.

Introduction
Feeding strategies to improve performance and preserve health in pigs while minimizing the use of antibiotics is of great interest for the swine industry. Probiotics are non-pathogenic live microorganisms that, once ingested in adequate amounts, can improve the intestinal microbial balance and benefit the animal. The beneficial effects of probi-

1 Appreciation is expressed to Calpis Co., Ltd. (Tokyo, Japan), and Quality Technology International, Inc. (Elgin, IL) for microbial analysis and financial support.
2 Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

Kansas State University Agricultural Experiment Station and Cooperative Extension Service
otics on intestinal health include the development of a healthy microbiota, prevention of colonization by enteric pathogens, increase in digestive capacity, reduced intestinal pH, and improvement in gut immunity, maturation, and integrity. The strains of bacteria that are typically used as probiotics for livestock animals include *Lactobacillus* sp., *Bifidobacterium* sp., *Enterococcus* sp., and *Bacillus* sp. *Bacillus* sp. are Gram-positive spore-forming bacteria that are used as probiotics either as single strain or multi-strain preparations. Spores are considered stable during feed manufacturing and storage, and after ingested can germinate but not proliferate in the intestine.

Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial product based on viable spores of a non-genetically modified strain, *Bacillus subtilis* C-3102. In swine, *Bacillus subtilis* C-3102 has been fed as a growth promoter in nursery pigs and is thought to work by increasing the populations of the beneficial intestinal microflora, particularly the *Lactobacillus* sp. However, this product has not been directly administered to piglets while they are nursing to determine if improved nursing performance or fecal microflora can be observed. Therefore, the objective of the present study was to evaluate the effect of a daily oral dose of Calsporin on fecal microflora of nursing pigs.

**Procedures**

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State Swine Teaching and Research Center in Manhattan, KS. A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in the study. The average parity was 2.5. At d 110 of gestation, sows were weighed and individually housed in an environmentally-controlled and mechanically-ventilated farrowing house.

Sows and litters were randomly assigned to treatments based on farrowing date and parity. Piglets were cross-fostered within 24 h after birth to equalize litter size. Treatments began on d 2 after birth and continued until weaning at approximately d 19 of age. Therefore, d 2 and 19 of age corresponded to d 0 and 17 of the trial, respectively. The treatments consisted of either a placebo control (n = 14 litters) or supplementation of Calsporin (Calpis Co. Ltd., Tokyo, Japan) in the control suspension (n = 12 litters). The treatments were applied orally to individual piglets once daily at approximately 0700 h. Daily Calsporin dosage was 45.0 × 10⁶ CFU/mL, 77.5 × 10⁶ CFU/mL and 108.3 × 10⁶ CFU/mL for d 0 to 7, d 7 to 14, and d 14 to weaning, respectively, administered via 1 mL liquid suspension. The liquid suspension was based on a carrier (Headsstart, Animal Science Products, Inc., Nacogdoches, TX, US) alone or with Calsporin. The preparation of treatments consisted of dissolving the products in warm water while continuously mixing the solution with a magnetic stirrer. The solution was prepared

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immediately before use and given in one oral dose of 1 mL per piglet. Both control and
treatment suspensions were analyzed for quantification of *Bacillus subtilis* C-3102.

Sows were allowed ad libitum feed intake during lactation with daily feed delivered and
recorded by an electronic feeding system (Gestal Solo Feeders Jyga Technologies, Que-
bec City, Quebec, Canada). Sow BW was measured on d 2 post-farrowing and weekly
until weaning. Piglets were individually weighed on d 2 after birth and on a weekly basis
until weaning. Fecal scoring was conducted on d 2 after birth, and after 1 and 2 weeks
of treatment to categorize the consistency of the feces per litter using a numerical scale
from 1 to 5: 1, hard feces like pellet; 2, firm formed stool; 3, soft moist stool that retains
shape; 4, soft unformed stool; and 5, watery liquid stool.

Microbial analysis was performed on fecal samples pooled by litter. Fecal samples were
collected directly from the rectum of the piglets using mini cotton tip swabs on d 2
after birth, and after 1 and 2 weeks of treatment. Microbial analysis of fecal samples was
performed by culture method and quantification (log_{10} CFU/g) of Calsporin (*Bacillus
subtilis* C-3102), *Lactobacillus* sp., *Clostridium perfringens*, *Salmonella* spp., *Enterococcus* sp., *Enterobacteriaceae*, total *Bacillus* sp., total aerobes, and total anaerobes. Limit of
detection for Calsporin, *Salmonella* spp. and total *Bacillus* sp. was 2 × 10^2.

Data were analyzed using a linear mixed model. Treatment was included as fixed effect
and block was included as random effect. Litter was the experimental unit. Sow BW,
sow ADFI, piglet BW, piglet weight gain, and litter size were fitted assuming a nor-
mal distribution of the response variables. Mortality was fitted assuming a binomial
distribution. Fecal score and microbial analysis were analyzed as repeated measures.
Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.4, SAS
Institute Inc., Cary, NC). Results were considered significant at *P* ≤ 0.05.

**Results and Discussion**

Quantification of *Bacillus subtilis* C-3102 in the oral products that were provided
daily to the piglets revealed undetectable levels in the control, and 7.95 × 10^8 CFU/g,
10.40 × 10^8 CFU/g and 9.84 × 10^8 CFU/g in the Calsporin treatment for d 0 to 7, d 7
to 14, and d 14 to 17 of the trial, respectively.

While not a primary objective due to limited replication, sow and litter performance
was measured. There was no evidence for differences on sow BW (*P* > 0.085; Table 1)
or ADFI (*P* > 0.342) during lactation among treatments. For litter performance, no evi-
dence for differences was observed on piglet BW (*P* > 0.067) or weight gain (*P* > 0.163)
during lactation and no evidence for differences was observed on litter size (*P* > 0.060)
or mortality (*P* > 0.334) throughout the study. Fecal score was reduced from d 0 to 7
and increased from d 7 to 14 in both treatments, suggesting hardening of feces during
the first week and softening of feces on the second week of trial, respectively. However,
there was no evidence for differences on fecal score between control and Calsporin-
treated litters (*P* > 0.358; Table 1).

Microbial analysis revealed a change in the numbers of Calsporin (*Bacillus subtilis*
C-3102), total *Bacillus* sp., and total aerobes (*P* < 0.05; Table 2) in litters treated with
Calsporin. The detected level of Calsporin was affected by the interaction between treat-
ment and day ($P < 0.001$), where the number of *Bacillus subtilis* C-3102 was increased to a greater extent in litters supplemented with Calsporin compared to controls on d 7 and 14 of treatment. Similarly, an interaction between treatment and day ($P < 0.001$) showed the number of total *Bacillus* sp. was increased in litters supplemented with Calsporin compared to controls on d 7 and 14 of treatment, but not on d 0. Both the levels of Calsporin and total *Bacillus* sp. in the controls increased over time, whereas levels in the Calsporin-treated litters increased from d 0 to 7 and remained constant until d 14 ($P < 0.001$). The number of total aerobes was reduced in piglets treated with Calsporin for 14 d ($P < 0.026$) and decreased from d 0 to 14 in both control and Calsporin-treated piglets ($P < 0.001$).

The number of *Lactobacillus* sp. increased from d 0 to 7 and decreased from d 7 to 14 ($P < 0.001$) in both treatments, whereas the detected levels of *Enterococcus* sp., Enterobacteriaceae, and total anaerobes decreased from d 0 to 14 ($P < 0.001$) in both treatments. There was no evidence for differences in number of *Lactobacillus* sp., *Enterococcus* sp., Enterobacteriaceae, and total anaerobes between control and Calsporin-treated litters ($P > 0.05$). The detected level of *Clostridium perfringens* remained constant during the period of treatment, with no evidence for difference between treatments ($P > 0.331$). The analysis revealed non-detectable levels of *Salmonella* spp. in the feces (limit of detection $2 \times 10^2$ CFU/g), except for one sample on d 0 in the control treatment ($2.75 \times 10^7$ CFU/g).

In conclusion, daily supplementation of Calsporin to nursing pigs resulted in slight changes in fecal microflora by increasing total *Bacillus* sp. and decreasing total aerobes but did not influence fecal consistency.
Table 1. Effects of orally dosing piglets with Calsporin on sow and litter performance

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Calsporin</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow BW, lb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0</td>
<td>489.6 ± 11.7</td>
<td>510.9 ± 12.7</td>
<td>0.181</td>
</tr>
<tr>
<td>d 7</td>
<td>482.9 ± 10.1</td>
<td>503.9 ± 10.8</td>
<td>0.135</td>
</tr>
<tr>
<td>d 14</td>
<td>478.4 ± 10.5</td>
<td>502.5 ± 11.2</td>
<td>0.099</td>
</tr>
<tr>
<td>d 17</td>
<td>476.1 ± 9.7</td>
<td>498.5 ± 10.3</td>
<td>0.085</td>
</tr>
<tr>
<td>Sow feed intake, lb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 7</td>
<td>11.3 ± 0.5</td>
<td>12.0 ± 0.5</td>
<td>0.342</td>
</tr>
<tr>
<td>d 7 to 14</td>
<td>15.7 ± 0.4</td>
<td>16.0 ± 0.4</td>
<td>0.648</td>
</tr>
<tr>
<td>d 14 to 17</td>
<td>17.4 ± 0.6</td>
<td>17.2 ± 0.6</td>
<td>0.772</td>
</tr>
<tr>
<td>d 0 to 17</td>
<td>14.3 ± 0.4</td>
<td>14.6 ± 0.4</td>
<td>0.570</td>
</tr>
<tr>
<td>Piglet BW, lb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0</td>
<td>3.58 ± 0.09</td>
<td>3.38 ± 0.09</td>
<td>0.067</td>
</tr>
<tr>
<td>d 7</td>
<td>6.65 ± 0.17</td>
<td>6.51 ± 0.18</td>
<td>0.489</td>
</tr>
<tr>
<td>d 14</td>
<td>10.71 ± 0.29</td>
<td>10.31 ± 0.31</td>
<td>0.283</td>
</tr>
<tr>
<td>d 17</td>
<td>12.25 ± 0.35</td>
<td>11.93 ± 0.37</td>
<td>0.484</td>
</tr>
<tr>
<td>Piglet weight gain, lb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 7</td>
<td>3.06 ± 0.11</td>
<td>3.17 ± 0.12</td>
<td>0.510</td>
</tr>
<tr>
<td>d 7 to 14</td>
<td>4.06 ± 0.15</td>
<td>3.80 ± 0.16</td>
<td>0.163</td>
</tr>
<tr>
<td>d 14 to 17</td>
<td>1.52 ± 0.13</td>
<td>1.61 ± 0.14</td>
<td>0.555</td>
</tr>
<tr>
<td>Total</td>
<td>8.67 ± 0.30</td>
<td>8.59 ± 0.33</td>
<td>0.849</td>
</tr>
<tr>
<td>Litter size, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0</td>
<td>16.0 ± 0.22</td>
<td>15.7 ± 0.23</td>
<td>0.310</td>
</tr>
<tr>
<td>d 7</td>
<td>15.7 ± 0.22</td>
<td>15.1 ± 0.23</td>
<td>0.060</td>
</tr>
<tr>
<td>d 14</td>
<td>14.9 ± 0.22</td>
<td>14.8 ± 0.23</td>
<td>0.803</td>
</tr>
<tr>
<td>d 17</td>
<td>14.8 ± 0.25</td>
<td>14.7 ± 0.27</td>
<td>0.923</td>
</tr>
<tr>
<td>Mortality, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 7</td>
<td>1.79 ± 0.88</td>
<td>3.72 ± 1.38</td>
<td>0.445</td>
</tr>
<tr>
<td>d 7 to 14</td>
<td>5.00 ± 1.47</td>
<td>1.66 ± 0.95</td>
<td>0.334</td>
</tr>
<tr>
<td>d 14 to 17</td>
<td>0.96 ± 0.67</td>
<td>0.56 ± 0.56</td>
<td>0.738</td>
</tr>
<tr>
<td>Total</td>
<td>7.47 ± 1.88</td>
<td>5.79 ± 1.79</td>
<td>0.512</td>
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<tr>
<td>Fecal score&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0</td>
<td>2.08 ± 0.20</td>
<td>2.11 ± 0.22</td>
<td>0.943</td>
</tr>
<tr>
<td>d 7</td>
<td>1.66 ± 0.14</td>
<td>1.52 ± 0.15</td>
<td>0.500</td>
</tr>
<tr>
<td>d 14</td>
<td>2.08 ± 0.20</td>
<td>2.36 ± 0.22</td>
<td>0.358</td>
</tr>
</tbody>
</table>

1 A total of 26 sows (DNA 241, DNA genetics) were used in a nursing trial with 14 sows for control and 12 sows for Calsporin treatment. Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial based on viable spores of *Bacillus subtilis* C-3102. Pigs were given one daily oral dose of Calsporin from d 0 to 17, for a total of 17 days of treatment. Day 0 corresponded to day 2 after birth and d 17 corresponded to the day of weaning at ~19 days of age. Calsporin dosage was 45 × 10<sup>6</sup> CFU/mL, 77.5 × 10<sup>6</sup> CFU/mL and 108.3 × 10<sup>6</sup> CFU/mL for d 0 to 7, d 7 to 14 and d 14 to wean, respectively. Values are mean ± standard error of the mean.

2 Consistency of the piglet feces per litter using a numerical scale from 1 to 5: 1, hard feces like peller; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool.
Table 2. Effects of orally dosing piglets with Calsporin on fecal microflora

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Control</th>
<th>Calsporin</th>
<th>Treatment</th>
<th>Day</th>
<th>Ttr × Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 0</td>
<td>d 7</td>
<td>d 14</td>
<td>d 0</td>
<td>d 7</td>
</tr>
<tr>
<td>Calsporin</td>
<td>2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.06</td>
<td>0.10</td>
<td>0.08</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Detected/sampled</td>
<td>2/14</td>
<td>7/14</td>
<td>14/14</td>
<td>7/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Total Bacillus sp.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.13</td>
<td>0.10</td>
<td>0.12</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Detected/sampled</td>
<td>10/14</td>
<td>14/14</td>
<td>14/14</td>
<td>11/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>7.84</td>
<td>8.85</td>
<td>8.48</td>
<td>8.04</td>
<td>8.84</td>
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<tr>
<td>SEM</td>
<td>0.16</td>
<td>0.06</td>
<td>0.10</td>
<td>0.19</td>
<td>0.06</td>
</tr>
<tr>
<td>Detected/sampled</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>11/11</td>
<td>12/12</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>8.74</td>
<td>8.79</td>
<td>8.59</td>
<td>8.72</td>
<td>8.84</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.13</td>
<td>0.15</td>
<td>0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>Detected/sampled</td>
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<td>14/14</td>
<td>14/14</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>8.58</td>
<td>7.59</td>
<td>5.41</td>
<td>8.74</td>
<td>7.25</td>
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<tr>
<td>SEM</td>
<td>0.11</td>
<td>0.19</td>
<td>0.52</td>
<td>0.11</td>
<td>0.21</td>
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<tr>
<td>Detected/sampled</td>
<td>13/13</td>
<td>14/14</td>
<td>12/14</td>
<td>10/10</td>
<td>12/12</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>9.20</td>
<td>8.33</td>
<td>6.97</td>
<td>9.05</td>
<td>8.34</td>
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<tr>
<td>SEM</td>
<td>0.10</td>
<td>0.09</td>
<td>0.27</td>
<td>0.11</td>
<td>0.10</td>
</tr>
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<td>Detected/sampled</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Total Aerobes</td>
<td>9.32</td>
<td>8.64</td>
<td>8.41</td>
<td>9.28</td>
<td>8.42</td>
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<tr>
<td>SEM</td>
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<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Detected/sampled</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Total Anaerobes</td>
<td>9.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.08</td>
<td>0.06</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Detected/sampled</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>12/12</td>
<td>12/12</td>
</tr>
</tbody>
</table>

1 A total of 26 litters were used in a nursing trial with 14 litter for control and 12 litter for Calsporin treatment. Pigs were given one daily oral dose of control or Calsporin from d 2 until d 19 (weaning), corresponding to d 0 until 17 of treatment, respectively. Calsporin dosage was 45 × 10<sup>6</sup> CFU/mL, 77.5 × 10<sup>6</sup> CFU/mL and 108.3 × 10<sup>6</sup> CFU/mL for d 0 to 7, d 7 to 14 and d 14 to wean, respectively. Fecal samples were collected directly from the rectum of the piglets on d 0, 7 and 14 after the beginning of treatment. Values are mean ± standard error of the mean. Units are log<sub>10</sub> CFU/g. The superscripts <sup>a,b</sup> indicate significant difference among days within each treatment and <sup>1,2</sup> indicate significant difference between treatments within each day.

2 Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial based on viable spores of Bacillus subtilis C-3102.

3 Limit of detection for Calsporin and total Bacillus sp. was 2 × 10<sup>2</sup> CFU/g.