Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (Bacillus subtilis C-3102)

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ABSTRACT: This field study assessed the efficacy of a probiotic based on viable spores of Bacillus subtilis C-3102 (Calsporin; Calpis Co. Ltd., Japan) on the health status and productivity of sows and their litters through 2 full, sequential reproductive cycles from service of the first cycle to weaning of the second cycle. Fifty-six sows were allocated to 2 experimental groups, an untreated control (T1) group and a probiotic-treated (T2) group that received the same basal feed as the T1 group plus the probiotic at an approximate allowance of 30 g/t of feed (3 × 10^5 cfu/g). The offspring of T1 and T2 sows were offered basal and T2 creep feed (3 × 10^5 cfu/g), respectively. Health and zootechnical parameters of sows and piglets were recorded. Feeding the probiotic to sows and piglets resulted in significant benefits, observed in both cycles: 1) improved sow body condition during pregnancy (P < 0.05), 2) increased sow feed consumption, 3) reduced sow weight loss during lactation (P < 0.05), 4) reduced sow weaning–estrus interval (P < 0.05), and 5) higher BW of piglets at weaning (P < 0.05). Additionally, a significant (P < 0.05) improvement in piglet birth weight and in the number of piglets weaned was observed in the second cycle of T2 sows, while a significant improvement of mean daily gain of piglets from birth to weaning was observed in the first cycle of T2 sows. Microbiological examination of fecal samples showed that probiotic treatment significantly reduced both Escherichia coli and Clostridium spp. in piglet feces, particularly during the second cycle. The data suggested that continuous feed supplementation with the probiotic is beneficial for both sows and piglets, since zootechnical benefits were observed in both cycles.

Key words: Bacillus, growth, health, piglet, probiotic, sows

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doi:10.2527/jas2014-7651

INTRODUCTION

During reproduction, a sow is subjected to many stress factors (service, changes of housing, mixing with other sows, gestation, lactation, weaning, etc.) as well as extreme physical changes, such as BW loss (Kranendonk et al., 2007; Spoolder et al., 2009). Body weight loss during lactation influences the subsequent reproductive cycle (Noblet et al., 1990), while nutrient absorption during gestation and lactation influences the number of piglets born alive, the number of stillborn piglets, and the BW of piglets at birth and weaning (Kranendonk et al., 2007). Enteric nutrient absorption as well as local immunity requires optimal balance among indigenous gut microflora (Sekirov et al., 2010). Such a balance may be achieved by providing probiotics, for example, live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host (Fuller, 1992).

Among various species of probiotic microorganisms, Bacillus spp. are distinguished by their ability to withstand adverse conditions of feed processing.
(e.g., pelleting; Soccol et al., 2010). Certain bacillary probiotics exert positive effects in pigs, such as higher weight gain, improved feed conversion ratios, and reduced incidence of postweaning diarrhea and mortality (Alexopoulos et al., 2001; Taras et al., 2005; Scharek et al., 2007; Schierack et al., 2007). In previous studies, some beneficial effects of bacillary probiotics in sows and litters were investigated over short- or medium-term periods (e.g., from 14 d or 17 wk before farrowing until weaning; Alexopoulos et al., 2001, 2004; Taras et al., 2005; Schierack et al., 2009).

The aim of this field study was to investigate the efficacy of Bacillus subtilis C-3102 on the health status and performance of sows and litters during 2 consecutive reproductive cycles (i.e., each cycle consisting of 1 gestation plus 1 lactation period). Hence, the effects of the probiotic could be assessed on the same animals after medium-term (1 cycle) and longer-term (2 cycles) treatments.

**MATERIALS AND METHODS**

**Good Clinical Practice and Good Laboratory Practice**

All work during this study was performed according to Good Clinical Practice for the Conduct of Clinical Trials Guidelines (July 2001) and Good Laboratory Practice Guidelines (Directive 2001/82/EC; Directive 2004/9/EC; Directive 2004/10/EC). The specifications of the trial satisfied all welfare needs of the animals with regard to feed, water, space, and treatments according to Good Farming Practice Guidelines (Directive 2010/63/EC; Commission recommendation 2007/526/EC).

**Feed Additive Tested**

The probiotic used in this study is based on viable spores of B. subtilis C-3102 (Calsporin; Calpis Co. Ltd., Tokyo, Japan). The species B. subtilis is often used to produce fermented soy foods such as natto or in other food fermentation processes and as producer strains for many food and feed enzymes. Bacillus subtilis C-3102 is used widely around the world as an animal feed additive and was approved in the European Union as a zootechnical feed additive (gut flora stabilizer) in broilers, weaned piglets, and turkeys in 2006, 2010, and 2011, respectively.

**Trial Farm**

The study was performed from March 2011 to February 2012 on a commercial farrow-to-finish pig farm in Greece, with a breeding stock of 350 PIC sows (Pig Improvement Company, Hendersonville, TN). The farm practiced all-in–all out flow and AI. It had its own feed mill, a farrowing house (with 7 rooms, each of 14 crates), and a nursery, a grower, a finisher, and a breeding–gestation building.

All farrowing rooms were automatically air conditioned/ventilated, keeping the temperature between 20 and 25°C. A common outer aisle connected the rooms in the farrowing house, and foot dips outside each room were used before personnel entered. The rooms were equipped with commercial crates for each sow including a creep area for her piglets. The sow area had a slatted floor, and the creep area had a concrete one. All farrowing crates were also equipped with nipple drinkers and with separate feeders for sows and piglets. Extra heat was provided to piglets from birth until 10 to 15 d of age, using infrared electric lamps placed in the creep area. Each farrowing crate was cleaned daily and, when vacant, thoroughly washed and disinfected. Introduction of late gestation sows to the room was never earlier than 2 to 3 d after disinfection. Each week, a new batch of prepartum sows filled a single room and remained there until weaning of their litters.

At weaning, at approximately 28 d postfarrowing, all piglets in a farrowing room were moved to the nursery building, whereas their dams were moved to the breeding–gestation building and placed separately in individual stalls, each with an automatic feed dispenser. Sows were monitored daily for signs of estrus. At estrus, each sow was subjected to double insemination with fresh semen (12 and 24 h after the detection of heat by a teaser boar).

The sows remained in individual stalls for approximately 30 d until pregnancy testing, after which pregnant sows were housed in gestation pens holding 14 to 15 sows. Each gestation pen had a front side of 15 separate feeding–resting cubicles with an equal number of automatic feeders, providing a daily, individual, preweighed feed ration to each pregnant sow. Each week, and approximately 7 d before farrowing, all sows in the gestation pen were moved to the farrowing building and allocated to individual farrowing crates to fill a single room. Cross-fostering was permitted only within 24 h after farrowing and within the same treatment group. No castration of male pigs was practiced.

Breeding animals were vaccinated against pseudorabies virus, parvovirus, erysipelas, atrophic rhinitis, Escherichia coli and Clostridium perfringens types A and C, and porcine reproductive and respiratory syndrome virus (modified live vaccine) infection. Piglets were also vaccinated against Mycoplasma hyopneumoniae and porcine circovirus type 2. For the control of endoparasites and ectoparasites, all adults were treated with ivermectin twice a year.
**Feeds and Feeding**

Gestation and lactation feeds (Table 1) were daily offered to each sow according to the following feeding schedule: 1) Gestation feed was offered from weaning to service day, ad libitum; from service to the 64th day of gestation, at 2.2 kg; from the 65th day of gestation to farrowing, at 2.8 kg; and, on day of farrowing, no feed. During the gestation period, sows were scored for body condition using a scale ranging from 1 (thin) to 5 (obese), with optimum condition scored at 3 (Young and Aherne, 2005). Those scoring 1 or 2 (thin sows) were supplemented with 0.5 kg/d extra feed during the first half of gestation, until they scored 3 (usually after 15–30 d). 2) Lactation feed was offered to each sow during the first 7 d of lactation and was gradually increased to a maximum of 2.5 kg plus 0.5 kg per suckling piglet, which was maintained until weaning. Creep feed was offered to piglets ad libitum from the eighth day of age to weaning (Table 1).

All feeds were presented in mash form; were based on corn, wheat, barley, and soya; and were free of antimicrobials, performance enhancers, other probiotics, or acidifiers for a period of 2 mo before the start until the end of the trial.

**Experimental Design**

Four sequential weekly batches of sows were treated and observed for a full reproductive cycle (from service through 1 pregnancy and 1 lactation period). Sows that were not culled or showed signs of infertility remained in the same treatment group, managed as described for cycle 1, and observed for a second reproductive cycle (Fig. 1).

There were 2 treatments. The untreated control (T1) treatment consisted of 2 batches of sows offered basal feeds that were top dressed daily with a T1 (placebo) premixture during the entire study period. The probiotic-treated (T2) treatment used 2 batches of sows offered basal feeds that were top dressed daily with T2 (probiotic) premixture, so that an allowance of $3 \times 10^5$ cfu/g final feed had been achieved. Piglets were offered the same basal creep feed ad libitum, but the T2 creep feed contained 30 g probiotic/t ($3 \times 10^5$ cfu/g final feed).

Each dam with her litter represented an experimental unit. Two treatment groups, each of 28 dams and their piglets, were formed at study start. The 2 groups of sows had a similar age and parity composition. During the second reproductive cycle, in a crossover design, T1 sows were housed in the same farrowing rooms previously used by T2 sows, and vice versa (Fig. 1).

Both T1 and T2 top dressing premixtures (sows) and feeds (creep) were tested 3 wk before use to confirm the absence or presence of *B. subtilis* C-3102 spores, respectively. Proximate analysis was used to determine protein, fat, fiber, ash, and moisture, according to official methods of the Association of Official Analytical Chemists (1990).

**Biosecurity Measures**

Probiotic-free sow feeds were manufactured in the feed mill of the trial farm. Premixtures and creep feeds were manufactured in a premixure factory, in treatment sequence from the T1 to T2, and then shipped in color-coded 20-kg bags to the trial farm. Different scoops for feeding were used in the order T1 to T2. During the breeding–gestation period, top dressing premixtures were individually placed in appropriate quantities in the automatic feed dispenser of each sow during the previous day.

In the farrowing house, separate foot dips and a separate cleaning set (clothing, boots, and shovels) and feeding kit (scoops and barrows) were used for each room.

In the breeding–gestation house, where airborne spread of probiotic microorganisms was possible, barn crews first attended to the T1 sows and then to the T2 sows, using proper disinfection and foot dips when necessary.

**Parameters Recorded and Calculated**

The following data were recorded—always by the same person—for the sows:

- Identification number and parity
- Dates of service, farrowing, weaning, postweaning estrus, or return to estrus
- Body weight and back fat at entry to and exit from the farrowing crate. Back fat thickness (the 2 upper fat layers) was measured using a Renco Lean Meater ultrasound device, following the manufacturer’s guidelines (Renco Corporation, Minneapolis, MN). Essentially, the average of 2 measurements 6.5 cm left and right from the backbone midline at the last rib level was calculated.
- The proportion of sows of each group supplemented for more than 15 d with extra gestation

### Table 1. Proximate analysis of sow pregnancy, lactation, and creep feeds

<table>
<thead>
<tr>
<th>Content</th>
<th>Pregnancy feed</th>
<th>Lactation feed</th>
<th>Creep feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>87.01</td>
<td>87.44</td>
<td>90.00</td>
</tr>
<tr>
<td>CP, %</td>
<td>13.64</td>
<td>16.74</td>
<td>22.00</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.21</td>
<td>4.58</td>
<td>5.00</td>
</tr>
<tr>
<td>Fiber, %</td>
<td>6.33</td>
<td>3.88</td>
<td>2.50</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.18</td>
<td>5.29</td>
<td>7.00</td>
</tr>
<tr>
<td>DE (MJ/kg)</td>
<td>14.5</td>
<td>14.0</td>
<td>16.5</td>
</tr>
</tbody>
</table>
Feed to achieve optimal body condition (score 3) at the end of pregnancy period

Feed consumed during lactation. Sow daily feed intake was calculated from the amount of feed offered daily according to the previously described feeding program minus the remaining amount of feed in the feeder on the following morning (feed weigh back method).

Proportion of sows in each group showing estrus
Proportion of sows in each group culled after the first cycle
Culling, mortality, and health: daily records, including probable causes of any culls, illness, or deaths
Any necessary treatment was individually applied

The following data were recorded for the litters—always by the same person:
Number and individual weights of newborn and weaned piglets and their gender
Creep feed intake
A diarrhea score was calculated on litter basis after a daily monitoring of each litter using a scale from 0 to 3 (0 = no diarrhea, 1 = slight, 2 = middle, and 3 = abundant). Then, all partial scores were added per pen for all days of lactation. Scoring was always made by the same person. Treatment of diarrheic/sick pigs was similar (spectinomycin for diarrheas or enrofloxacin for more severe illness).
Mean daily gain of piglets

Sampling and Examinations

Rectal fecal samples from all sows on gestation Day 0 (before probiotic administration), 15, 60, and 110, and at weaning were collected, labeled, and stored in the refrigerator at 4°C. In addition, random rectal fecal samples were collected from 5 litters (3–4 piglets per litter) per group on lactation Day 8 and at weaning. All available samples were processed the next day for *E. coli*, *Bacillus* spp., and *Clostridium* spp.

For bacteriological analysis of fecal samples, 10-g samples were weighed aseptically, placed into sterile stomacher bags, and homogenized for 2 min in 90 mL of peptone water.

For the isolation of *E. coli*, 1-mL aliquots of 6 serial 10-fold dilutions of the homogenized samples were cultivated on tryptone bile x-glucuronide agar (92435 FLUKA; Sigma-Aldrich, Steinheim, Germany), according to ISO 16649-2:2001. All blue colony types were enumerated. The dilutions were used so that the

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**Cycle 1**

Gestation building - Individual stalls (from piglet weaning to service; from service to pregnancy confirmation - Trial start)

<table>
<thead>
<tr>
<th>Sow batch 1 - T1 placebo</th>
<th>Sow batch 2 - T1 placebo</th>
<th>Sow batch 3 - T2 Calsporin</th>
<th>Sow batch 4 - T2 Calsporin</th>
</tr>
</thead>
</table>

Gestation building - Gestation pens of 15 sows with individual feeding cubicles (from 0 to 106 d of gestation)

<table>
<thead>
<tr>
<th>Sow batch 1 - T1 placebo (gestation pen 1) - 14 sows</th>
<th>Sow batch 2 - T1 placebo (gestation pen 2) - 14 sows</th>
<th>Sow batch 3 - T2 Calsporin (gestation pen 3) - 14 sows</th>
<th>Sow batch 4 - T2 Calsporin (gestation pen 4) - 14 sows</th>
</tr>
</thead>
</table>

Farrowing building - Rooms with individual farrowing crates (from 107 d of gestation to weaning)

<table>
<thead>
<tr>
<th>Sow batch 1 - T1 placebo (farrowing room 1) - 14 sows with their litters</th>
<th>Sow batch 2 - T1 placebo (farrowing room 2) - 13 sows with their litters</th>
<th>Sow batch 3 - T2 Calsporin (farrowing room 3) - 14 sows with their litters</th>
<th>Sow batch 4 - T2 Calsporin (farrowing room 4) - 14 sows with their litters</th>
</tr>
</thead>
</table>

**Cycle 2**

Gestation building - Individual stalls (from piglet weaning to service; from service to pregnancy confirmation)

<table>
<thead>
<tr>
<th>Sow batch 3 - T2 Calsporin</th>
<th>Sow batch 4 - T2 Calsporin</th>
<th>Sow batch 1 - T1 placebo</th>
<th>Sow batch 2 - T1 placebo</th>
</tr>
</thead>
</table>

Gestation building - Gestation pens of 15 sows with individual feeding cubicles (from 0 to 106 d of gestation)

<table>
<thead>
<tr>
<th>Sow batch 3 - T2 Calsporin (gestation pen 1) - 11 sows</th>
<th>Sow batch 4 - T2 Calsporin (gestation pen 2) - 12 sows</th>
<th>Sow batch 1 - T1 placebo (gestation pen 3) - 12 sows</th>
<th>Sow batch 2 - T1 placebo (gestation pen 4) - 9 sows</th>
</tr>
</thead>
</table>

Farrowing building - Rooms with individual farrowing crates (from 107 d of gestation to weaning)

<table>
<thead>
<tr>
<th>Sow batch 3-T2 Calsporin (farrowing room 1) - 11 sows with their litters</th>
<th>Sow batch 4-T2 Calsporin (farrowing room 2) - 12 sows with their litters</th>
<th>Sow batch 1-T1 placebo (farrowing room 3) - 12 sows with their litters</th>
<th>Sow batch 2-T1 placebo (farrowing room 4) - 9 sows with their litters</th>
</tr>
</thead>
</table>

**Figure 1.** Experimental layout. T1 = untreated control; T2 = probiotic treated. The replacement sows (e.g., up to the number of 14 sows) with their litters were excluded from the trial.
number on 1 plate was countable, for example, minimum 10 colonies and maximum 300 colonies were counted per plate (equals 10 to 300 cfu/plate). The same was used also for Clostridium spp.

For the isolation of Clostridium spp., 1-mL aliquots of 6 serial 10-fold dilutions of the homogenized samples were mixed with 9 mL of sulfite-polymyxin-sulfadiazine agar (85627 FLUKA; Sigma-Aldrich). After 18 h of anaerobic incubation, sulfite-reducing Clostridium cells were counted. Colonies surrounded by the characteristic black precipitate were biochemically identified by using lactose fermentation, nitrate reduction, gelatinase production, and motility tests.

For the isolation of Bacillus spp., 1-mL aliquots of 6 serial 10-fold dilutions of the homogenized samples were plated in duplicate directly on HiCrome Bacillus Agar (92325 FLUKA; Sigma-Aldrich) and incubated at 30 and 37°C, respectively. Furthermore, heat treatment of fractions of the fecal samples (80°C for 15 min) was performed before dilution and plating on HiCrome Bacillus Agar.

Statistical Analysis

In this study, the experimentalist took the view that the most appropriate unit is the smallest unit on which a measurement was made (in this case the animal). During the experiment, each animal was individually fed; hence, the appropriate experimental unit would be individual sow and her litter (Robinson et al., 2006). Both parametric and nonparametric statistical methods were applied for the statistical evaluation of the experimental results. In case of normality and variance’s homogeneity, 1-way ANOVA was performed to evaluate possible significant effects of the probiotic on sow and litter performance in both reproductive cycles. Where assumptions about either variability or the form of populations distribution were seriously violated, with or no transformed data, the nonparametric Wilcoxon rank sum test (Mann–Whitney U-test) was applied to evaluate treatment differences. A repeated measures ANOVA was performed in cases where “time” was included as a repeated measure in the statistical analysis (sows’ BW and back fat measurements and fecal samples). All analyses were conducted using the statistical software program SPSS for Windows (version 20.0 for Mac OS X, 1989–2011; SPSS Inc., Chicago, IL). Significance was declared at $P \leq 0.05$, unless otherwise noted. Back-transformed mean values are reported in the results.

RESULTS

The effect of the probiotic on sow performance during 2 successive reproductive cycles is presented in Table 2. During the study, 1 T1 sow died from splenic torsion immediately after her first farrowing, so her piglets were cross-fostered on other T1 sows. At the end of the first reproductive cycle, the same sows were followed over their second reproductive cycle, except those that were removed from the study. One T1 sow was culled due to leg trauma and another 5 T1 sows were removed because of anestrus, whereas from the T2 group, 3 sows were removed because of lameness and sickness and another 2 because of anestrus. Neither anestrus nor culling rates were significantly different between treatment groups ($P > 0.05$). Therefore, at the start of the second reproductive cycle, the T1 group included 21 sows and the T2 group included 23 sows.

Mean sow parity did not significantly differ between the groups in either cycle (Table 2). The proportion of sows that received extra feed for at least 15 d of their pregnancy was significantly higher in T1 compared to T2 sows in both cycles (81.5 and 53.6% in the first cycle and 81.0 and 39.1% in the second cycle, respectively; $P < 0.05$). By entry to the farrowing rooms, sow body condition was score 3 in both groups, and no significant differences were observed between treatments in sow BW (Table 2) and back fat measurements (data not shown). However, at the end of the lactation period in both cycles, significantly higher BW losses were observed in T1 over T2 sows ($P < 0.05$; Table 2). Whereas T1 sows lost 19.6 and 16.8% of their initial BW by the first and second weanings, respectively, the T2 sows lost 14.5 and 10.6%, respectively, over the same period. Consumption of lactation feed was higher in the T2 sows compared to T1 sows during the 2 lactation periods ($P < 0.05$; Table 2). Finally, whereas no differences were observed between treatments in the proportion of sows showing estrus or (data not shown), there was a significantly shorter weaning–estrus interval in the T2 sows versus T1 sows in both reproduction cycles ($P < 0.05$).

Counts of B. subtilis C-3102 in sow feces confirmed that, during all reproduction stages in both cycles (except 1 case at gestation Day 15 of the first cycle), cross-contamination between groups with the testing material did not occur; that is, T1 sows remained essentially uncontaminated by B. subtilis C-3102. On the other hand, feces from T2 sows were positive during the entire study period with counts ranging from $6.2 \times 10^2$ to $1.7 \times 10^3$ cfu/g feces. Furthermore, no significant differences in fecal counts of E. coli and Clostridium spp. were observed between T1 and T2 sows, except a significant reduction of fecal E. coli at 110 d of gestation in the second cycle of T2 sows ($P < 0.05$).

The effects of probiotic administration on litter performance during 2 successive reproductive cycles are presented in Table 3. The total number and BW of
piglets born were not significantly different between the T1 and T2 groups in both cycles. No significant differences in neonatal mortality or fecal scores were observed between the T1 and T2 groups either cycle or in creep feed consumption. The number of weaned piglets during the first cycle was not different between the T1 and T2, but in the second cycle, T2 sows weaned significantly more piglets than T1 sows ($P < 0.05$). The mean weight of T2 piglets at weaning was approximately 0.5 kg higher than T1 piglets in both cycles ($P < 0.05$). Finally, the mean daily gain of T2 piglets was higher than T1 piglets in both cycles but significantly so only during the first cycle.

With regard to fecal microbiology, T1 piglets remained uninfected by *B. subtilis* C-3102 in both cycles, indicating absence of cross-contamination between groups. In contrast, *B. subtilis* C-3102 spores were detected in low numbers (2 $\times 10^1$ to $1.6 \times 10^3$ cfu/g feces) in some T2 piglets from 8 d of age. A tendency for reduced *Clostridium* spp. in T2 piglet feces at 8 d of age during the first cycle ($1.17 \times 10^3$ cfu in T2 vs. $1.29 \times 10^4$ cfu in the T1; $P = 0.07$) and a significant reduction of *E. coli* ($3.38 \times 10^3$ cfu in T2 vs. $5.06 \times 10^4$ cfu in the T1) and *Clostridium* spp. ($2.70 \times 10^2$ cfu in T2 vs. $3.25 \times 10^3$ cfu in the T1) in fecal samples were also observed in T2 piglets at 8 d of age in the second cycle ($P < 0.05$).

**DISCUSSION**

Several probiotics have shown potential benefits in animals and humans, including effects on the gut-associated and systemic immunity (Isolauri et al., 2001; Schultz et al., 2003; Mack and Lebel, 2004; Sekirov et al., 2010). The purpose of this study was to conduct a field investigation on the efficacy of *B. subtilis* C-3102 on the health and performance of sows and their litters. Bacillary probiotics are known for their ability to withstand adverse conditions (e.g., feed pelleting, storage at ambient temperatures, survival at low pH), properties that make them good candidates for animal use (Nicholson et al., 2000; Silley, 2006). Not many studies have investigated the practical effects of probiotics on sows under commercial husbandry conditions and particularly after medium-term (1 cycle) or longer-term (2 cycles) administration, maintaining the same sows in each treatment over the entire study duration.

The study design included a crossover element (swapping T1 pens and T1 rooms with T2 pens/rooms during the second reproductive cycle) to minimize room/pen effects and improve the robustness of the data. Although appropriate measures were taken, cross-contaminations between treatments could not be excluded over such a long study period and taking into account the common housing and air space of dry sows. Nevertheless, the fecal data suggest that cross-contamination was low and infrequent (e.g., some sows on gestation Day 15 of the first cycle). Unless continually administered, probiotics rapidly disappear from the gut (Silley, 2006). Indeed, frequent fecal titrations of *B. subtilis* C-3102 in a substantial sample of animals at regular periods confirmed that cross-contamination between treatments was not a major issue.

Probiotic administration improved sow body condition in both pregnancy cycles, as fewer T2 sows needed supplementary feed to score 3 by the time of farrowing. The average BW of sows at the start of the first cycle, and specifically after pregnancy detection, was the same between groups (data not presented). Body weight was also similar between sow groups close to farrowing (Table 2). Apparently, this latter lack of difference was most likely due to the more intense feeding of control sows during precedent pregnancy to achieve score 3. Sow body condition at the end of pregnancy depends mainly on body condition at start of the pregnancy (i.e., just after the preceding weaning). Thereafter, sow condition may also depend on proper supply and use of nutrients during the various stages of pregnancy. Several studies have shown that probiotics may enhance the absorptive capacity of

**Table 2. Efficacy of in-feed *Bacillus subtilis* C-3102 in sows during 2 successive reproductive cycles (mean [SD])**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First cycle</th>
<th>Second cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1$^1$</td>
<td>T2$^2$</td>
</tr>
<tr>
<td></td>
<td>$n = 27$</td>
<td>$n = 28$</td>
</tr>
<tr>
<td>Parity</td>
<td>3.3 (1.3)</td>
<td>3.6 (2.2)</td>
</tr>
<tr>
<td>Lactation period, d</td>
<td>30.1 (1.2)</td>
<td>29.7 (1.4)</td>
</tr>
<tr>
<td>Sow weight at farrowing, kg</td>
<td>238.7 (30.7)</td>
<td>242.0 (25.9)</td>
</tr>
<tr>
<td>Back fat loss, mm</td>
<td>3.9 (1.3)</td>
<td>3.4 (1.9)</td>
</tr>
<tr>
<td>Sow weight loss, kg</td>
<td>46.9 (10.8)</td>
<td>35.0$^3$ (12.3)</td>
</tr>
<tr>
<td>Feed consumption, kg</td>
<td>219.1 (25.6)</td>
<td>236.9$^3$ (28.5)</td>
</tr>
<tr>
<td>Wean-estrus interval, d</td>
<td>6.5 (1.6)</td>
<td>5.3$^3$ (1.0)</td>
</tr>
</tbody>
</table>

$^1$T1 = untreated control.
$^2$T2 = probiotic treated.
$^3$Figures in same row, within same cycle, differ significantly ($P < 0.05$).
Performance of probiotic-treated sows

The performance of probiotic-treated sows was reduced only in T2 sows. The higher feed intake and weaning-to-estrus intervals compromise litter uniformity, independent of sow parity (Wientjes et al., 2013).

As nutrient utilization during pregnancy improves, so does fetal growth. On the other hand, better nutrient utilization during lactation is reflected in higher milk quality, which may partially explain the better growth and higher piglet weaning weights observed in this study. Such positive effects have been described by other researchers (Alexopoulos et al., 2004; Taras et al., 2005; Bohmer et al., 2006).

Piglets may receive probiotics either directly by their creep feed or indirectly from the sow and her environment, enriched by sow feces or feed spillage. Treated dams were excreting *B. subtilis* C-3102 during the entire administration period, and *B. subtilis* C-3102 spores were detected in piglet feces at 8 d of age, just before the piglets had access to treated creep feed. This suggests indirect uptake of probiotic spores from contact with the dam. Most likely, creep feed is not the most important way of acquiring probiotic during the early phases of suckling, because creep feed consumption before 15 d of age is low. Irrespective of how piglets acquired the probiotic, there are 2 hypothetical mechanisms whereby T2 piglets achieved higher weaning weights than T1 piglets, on the order of 0.5 kg. One might involve better utilization of nutrients derived either from sow milk or from creep feed. A second mode of action may be reduction of undesirable microbes in the piglet gut, evidenced in this study as lower fecal *E. coli* and *Clostridium* spp. counts in the early stages of the second cycle. Several studies have shown that probiotics exert positive enteric or systemic effects in piglets, either directly or by enhancing certain immune mechanisms (Shu et al., 2001; Jadamus et al., 2002; Scharek et al., 2005; Taras et al., 2005, 2006; Kritas and Morrison, 2007; Schierack et al., 2007, 2009; Konstantinov et al., 2008; Szabó et al., 2009; Kreuzer et al., 2012; Baker et al., 2013). Depending on the microbial species and the farm health status, a reduction in enteric pathogens may be either subclinical or clinically obvious, for example, lower mortality or less piglet di-

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### Table 3. Efficacy of in-feed *Bacillus subtilis* C-3102 in litters of 2 successive reproductive cycles (mean [SD])

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First cycle</th>
<th></th>
<th>Second cycle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>T2&lt;sup&gt;2&lt;/sup&gt;</td>
<td>T1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>T2&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of piglets born</td>
<td>12.26 (1.23)</td>
<td>12.32 (2.75)</td>
<td>12.24 (1.14)</td>
<td>12.61 (1.16)</td>
</tr>
<tr>
<td>Piglet birth weight, kg</td>
<td>1.58 (0.41)</td>
<td>1.55 (0.37)</td>
<td>1.51 (0.51)</td>
<td>1.47 (0.50)</td>
</tr>
<tr>
<td>Number of piglets weaned</td>
<td>11.19 (1.21)</td>
<td>11.18 (1.36)</td>
<td>11.10 (1.22)</td>
<td>11.87 (1.14)</td>
</tr>
<tr>
<td>Piglet weaning weight, kg</td>
<td>7.47 (1.65)</td>
<td>8.01&lt;sup&gt;3&lt;/sup&gt; (1.64)</td>
<td>7.66 (1.05)</td>
<td>8.05&lt;sup&gt;3&lt;/sup&gt; (1.24)</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>8.4 (10.1)</td>
<td>6.3 (6.1)</td>
<td>9.1 (8.6)</td>
<td>5.7 (6.0)</td>
</tr>
<tr>
<td>Diarrhea score</td>
<td>5.2 (5.1)</td>
<td>4.6 (2.1)</td>
<td>6.4 (5.2)</td>
<td>4.2 (3.2)</td>
</tr>
<tr>
<td>ADG, g</td>
<td>195.0 (27.7)</td>
<td>217.5&lt;sup&gt;3&lt;/sup&gt; (25.7)</td>
<td>236.1 (27.4)</td>
<td>244.0 (23.1)</td>
</tr>
</tbody>
</table>

<sup>1</sup>T1 = untreated control; piglets from untreated sows.<br><sup>2</sup>T2 = probiotic treated; sows from probiotic-treated sows.<br><sup>3</sup>Figures in same row, within same cycle, differ significantly (P < 0.05).
arrhea. The trial farm did not suffer from severe health problems and, therefore, no significantly clinical differences were evident. However, an adverse bacterial gut load may also be important with respect to production parameters, as energy spent fighting nonbeneficial bacteria is lost to the animal, and to the farmer, in terms of growth and efficient feed conversion.

In conclusion, the data from this study indicate that feed supplementation with *B. subtilis* C-3102 improves performance in both sows and piglets and that long-term feeding over several reproductive cycles may bring incremental health and production benefits. As each probiotic is based on distinct microbial strain or strains with unique properties and behavior, this is something that may explain differing results by other products. Further investigations will help to establish a solid understanding of the modes of action and effectiveness of various probiotic strains in animals.

**LITERATURE CITED**


