

## Effect of Direct Fed Microbial, *Bacillus subtilis* C-3102, on Enteric Health in Nursery Pigs After Challenge with Porcine Epidemic Diarrhea Virus (PEDv)

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Since Porcine Epidemic Diarrhea virus (PEDv) hit the USA and Canada in 2013, producers have been investigating interventions to reduce the impact of PEDv in their swine herds. Among those interventions of interest are direct fed microbials (DFMs) which may have role in improving gut health and growth in pigs. To date, there have been no peer-reviewed studies describing the effect of probiotics or direct fed microbials on intestinal health and pathology following challenge or exposure to PEDv. **In this bulletin, we examine the impact of feeding *Bacillus subtilis* C-3102 (CALSPORIN®) on gut health, inflammation and regeneration after a PEDv challenge in nursery pigs.**

### 1. Objective:

To examine the effects of feeding *Bacillus subtilis* C-3102 at the target inclusion rates of 0 colony forming units (CFU) per g, 500,000 CFU per g, and 1 million CFU per g on intestinal health in weaned pigs after challenge with porcine epidemic diarrhea virus (PEDv).

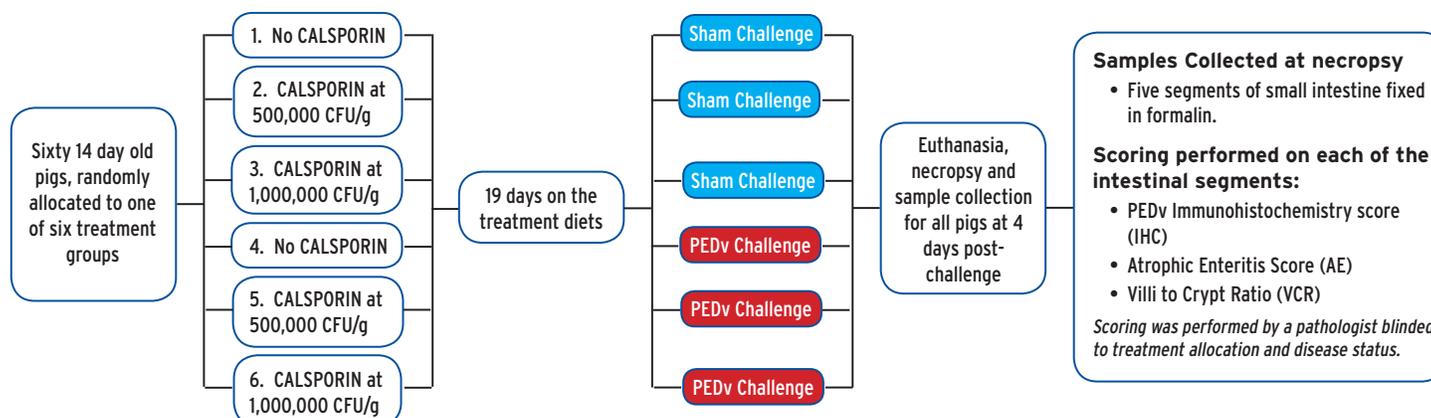
### Materials and Methods:

This was a 2×3 factorial design involving three experimental diets and PEDv or sham challenge (Figure 1). The experimental diets were standard, commercially prepared, antibiotic-free nursery pig diets containing *Bacillus subtilis* C-3102 (CALSPORIN at 0, 500,000, or 1 million CFU per g). Groups were challenged with either PEDv-positive or PEDv-negative cell-culture fluids by oral gastric gavage. Figure 1 displays the six treatment groups represented in this design. Sixty 14-day-old pigs, PEDv PCR negative pigs were randomly allocated into six treatment groups with 10 pigs per group (Figure 1).

The study diets were then fed for 19 days and pigs were inoculated with PEDv-positive or PEDv-negative cell-culture fluid on day 22 (Figure 1). Four days after inoculation (day 26), pigs were euthanized and necropsied. The pigs remained on the treatment diets up to and including the day of necropsy, totaling 23 days on the experimental diets.

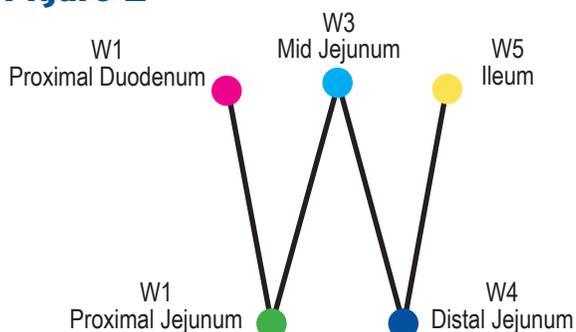


**Figure 1** - Schematic diagram of trial design and samples collected at necropsy.



At necropsy, five standardized segments of intestine were collected for PEDv immunohistochemistry (IHC), villus to crypt ratio (VCR) and atrophic enteritis scoring (AE) (Figure 2 and Table 2). Fecal samples were also collected for PEDv PCR. Measurements of animal growth performance were also collected (Figure 1).

**Figure 2**



Schematic diagram of the five intestinal segments collected on each pig. On each segment, PEDv immunohistochemistry (IHC), atrophic enteritis (AE) score and villi to crypt ratio (VCR) scoring was performed.

**Table 2** - Overview of scoring for intestinal health parameters on five segments per pig

Score	Interpretation
Immunohistochemistry Scoring (IHC)	<p><u>Scoring system</u></p> <p>Score 0=negative</p> <p>Score 1=&lt;10% of villous enterocytes within section showing a positive PEDv signal</p> <p>Score 2=10-50%</p> <p>Score 3=&gt;50%</p> <ul style="list-style-type: none"> <li>• Lower score means less PEDv present in the gut</li> </ul>
Villi: crypt ratio (VCR)	<ul style="list-style-type: none"> <li>• Measurement of intestinal damage and regeneration</li> <li>• Bigger VCR means healthier gut</li> </ul>
Atrophic Enteritis (AE) score	<ul style="list-style-type: none"> <li>• Scored based on severity and percentage of sample affected</li> <li>• Cellular damage to the intestine (intestinal inflammation)</li> <li>• Lower score indicates healthier gut</li> </ul>

**Table 3** - Least squares means estimates of 4 day average daily gain (ADG) for all treatment groups between the day of inoculation and day of necropsy. ADG reflects a total of 4 days. There was not a statistically significant difference between any groups.

Treatment Group	Estimate of ADG (kg)	Standard Error
1	0.2383	0.0390
2	0.4150	0.0390
3	0.3770	0.0390
4	0.0963	0.0390
5	0.1838	0.0390
6	0.1963	0.0390

## Results and Discussion:

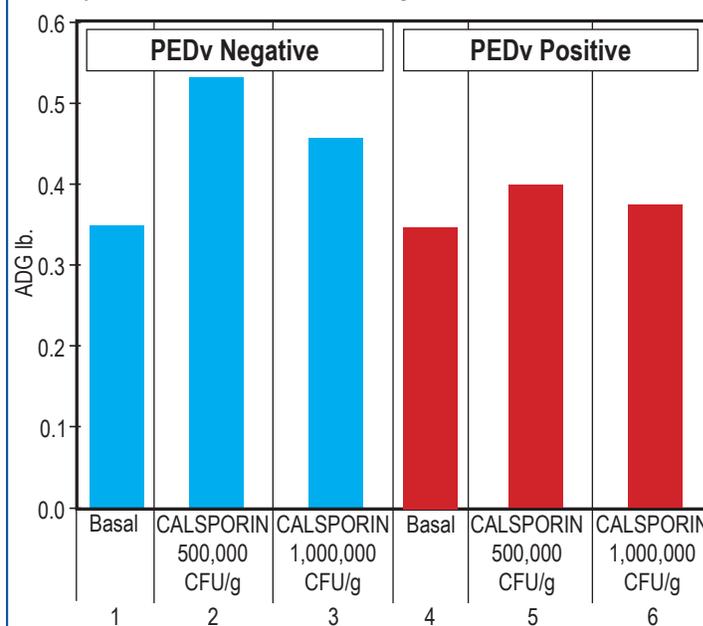
Pathological changes associated with PEDv were significantly less severe in PEDv challenged treatment groups that received *B subtilis* C-3102 than in the group that received no *B subtilis* treatment. Figures 3, 4 and 5 summarize the improvements in IHC, VCR and AE score seen in group 5 compared to group 4. There were no significant differences in small intestinal length, ratio of small intestinal weight to body weight, colon dry matter content, average daily gain, or fecal scoring between any of the six treatment groups. Average daily gain information is presented in Tables 3 and 4, although there were no statistically significant differences in ADG between groups.

**Table 4** - Least squares means estimates of 23 day average daily gain (ADG) for all treatment groups. ADG reflects total of 23 days representing the duration in which the pigs were exposed to the treatment diets. There was not a statistically significant difference between any groups.

Treatment Group	Estimate of ADG (kg)	Standard Error
1	0.1577	0.0179
2	0.2394	0.0179
3	0.2046	0.0179
4	0.1413	0.0179
5	0.1804	0.0179
6	0.1713	0.0179

## Average Daily Gain Results

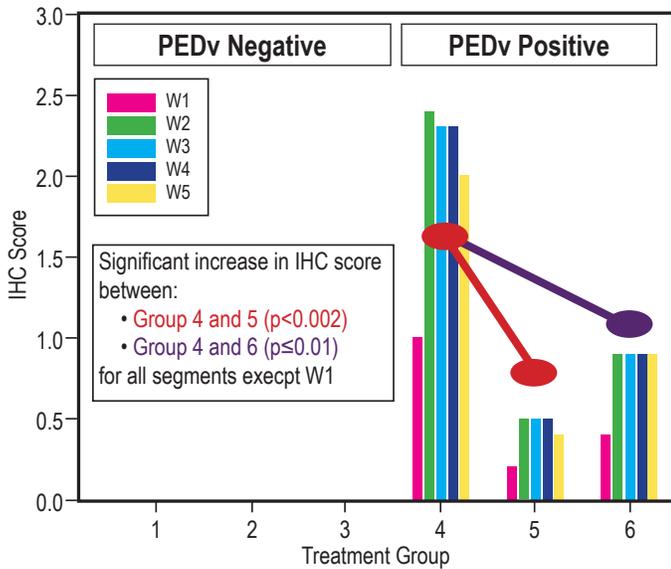
23 Days Continuous Treatment Feeding Results



## 2. Take Home Messages:

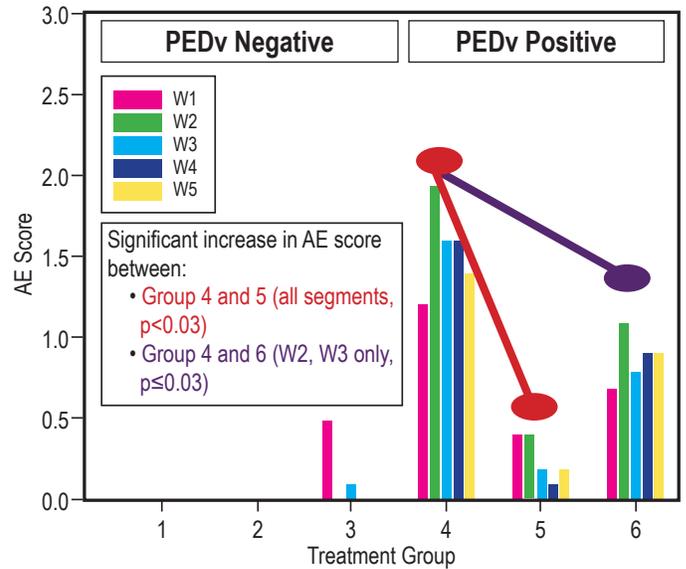
Under the conditions of this study, treatment with *B subtilis* C-3102 in nursery pigs challenged with PEDv was associated with better enteric health compared to pigs not treated with *B subtilis* C-3102.

**Figure 3**



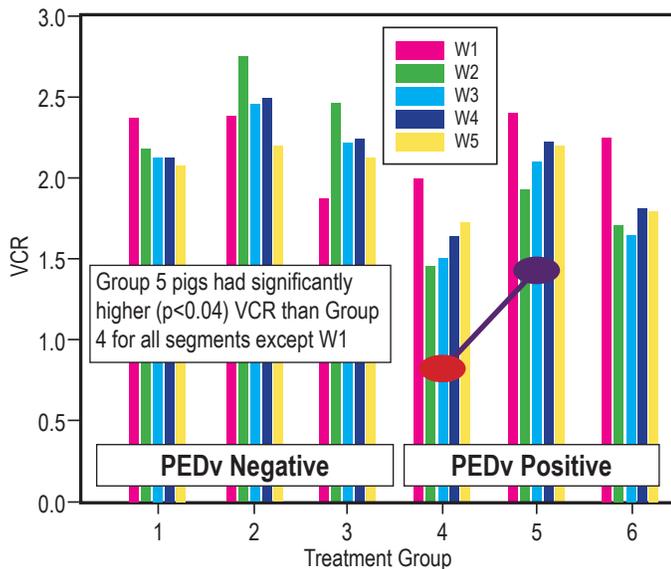
Least squares means estimates for Porcine Epidemic Disease virus (PEDV) immunohistochemistry (IHC) scores for each treatment group for each intestinal segment. The intestinal segments were as follows: duodenum (W1), proximal jejunum (W2), mid jejunum (W3), distal jejunum (W4), and ileum (W5). Treatment groups are described in table 1. For treatment groups 1, 2 and 3, the IHC score for all segments was zero. Comparisons among treatment groups were made using F-tests followed by Tukey's *t* tests for multiple comparisons.

**Figure 5**



Least squares means estimates for atrophic enteritis (AE) scores for each treatment group for each intestinal segment. The intestinal segments were as follows: duodenum (W1), proximal jejunum (W2), mid jejunum (W3), distal jejunum (W4), and ileum (W5). Treatment groups are described in table 1. Comparisons among treatment groups were made using F-tests followed by Tukey's *t* tests for multiple comparisons.

**Figure 4**



Least squares means estimates for villus height to crypt-depth ratios (VCR) for each treatment group for each intestinal segment. The intestinal segments were as follows: duodenum (W1), proximal jejunum (W2), mid jejunum (W3), distal jejunum (W4), and ileum (W5). Treatment groups are described in table 1. Comparisons among treatment groups were made using F-tests followed by Tukey's *t* tests for multiple comparisons.

**Citation:**

Canning P, Ruston C, Madson D, et al. Effect of direct-fed microbial *Bacillus subtilis* C-3102 on enteric health in nursery pigs after challenge with porcine epidemic diarrhea virus. *J Swine Health Prod.* 2017;25(3):129–137.

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