Exploring the use of Probicon L28 and BIOPLUS® 2B as direct-fed microbials to reduce *Salmonella* and Shiga toxin-producing *Escherichia coli* in market hogs

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Background

Market hogs are often a critical reservoir for *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC). Thus, these pathogens can be found in the environment of swine farms. *Salmonella* has also been recovered from market hog lymph nodes, which can pose a risk to pork products. Intervention application at the abattoir is important; however, a need exists for identifying an effective intervention(s) that reduces pathogens in market hogs pre-harvest to reduce the pathogen burden entering the abattoir. Probiotics, especially lactic acid bacteria, have been heavily researched in cattle as pre-harvest interventions, particularly for *E. coli* O157:H7 control. BIOPLUS® 2B, which is comprised of *Bacillus subtilis* and *Bacillus licheniformis*, is currently the most common probiotic used in the swine industry to establish microflora of the gastrointestinal tract and aid in healthy animal performance. *Lactobacillus salivarius* L28 (Probicon L28) is patented as a food safety intervention that is included in animal diets to reduce pathogens. Because there is a need for effective pre-harvest interventions in the swine industry, the objective of this study was to investigate BIOPLUS® 2B and Probicon L28 as pre-harvest interventions to reduce *Salmonella* and Shiga toxin-producing *Escherichia coli* in market hogs.

Two groups of pigs (group 1 n=294; group 2 n=356, initial BW = 116.2 lb) were enrolled in this study. For each group of pigs, a total of 36 pens were used (n=72 pens total), with 12 pens per treatment (n=24 pens total). Pigs were randomly assigned to pen, and pens were assigned to one of three treatments: a standard corn-SBM finishing diet with Probicon L28 supplemented through water lines using a Dosatron system at a target concentration of 1.0×10^6 CFU/head/day, a standard corn-SBM finishing diet supplemented with BIOPLUS® 2B (5.0×10^8 CFU/pound of feed; ~3.0×10^9 CFU/head/day), and a control treatment with the standard corn-SBM finishing diet, with no added probiotic. On three separate occasions throughout the feeding period (upon arrival/baseline, mid-way, and prior to loadout), pig pens were sampled. At each sampling period, feces were collected from 4 pigs at random. Pig feces (n=144 per sampling period, 10g) were added to 90 mL of BAX®MP medium with 0.5 mL/L of Quant solution (BAX®MP+0.5Quant) and 10 mL of feces homogenate (FH) was transferred into 10 mL of pre-warmed (42°C) BAX®MP+0.5Quant. All FH samples were incubated at 42°C for 24 hours. Market hogs were followed to the abattoir where superficial inguinal lymph nodes (SILNs) were collected. The SILN samples (group 1 n=262; group 2 n=314) were collected and trimmed of fat and fascia, weighed, boiled for 3-5

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sec, smashed, and homogenized with 80 mL of BAX®MP to prepare a lymph node homogenate (LNH). All LNH samples were incubated at 42°C for 24 hours. Following incubation, STEC and *Salmonella* were detected using the BAX® System Real-time STEC Screening Suite [STEC screen (*stx*, *eae* genes), Panel 1 (O26, O111, O121 serogroups), Panel 2 (O45, O103, O145 serogroups), *E. coli* O157:H7 EXACT] and BAX® System Real-Time *Salmonella* Assay, respectively.

Results

Salmonella prevalence was very low throughout this study. Feces collected from group 1 and group 2 market hogs had an overall *Salmonella* prevalence of 0.7% and 2.8%, and 0% and 0%, at the beginning and end of the feeding period, respectively. *Salmonella* prevalence in SILNs of group 1 and group 2 market hogs was 0.8% and 0%, respectively. In pig fecal samples, the prevalence of STEC (*stx* and *eae* genes) from control, BIOPLUS® 2B, and Probicon L28 market hogs was 10.0%, 5.1%, and 19.0%, respectively. In comparison to the control, there was no evidence that the DFM diets had an impact ($P>0.05$) on the prevalence of STEC or the O26, O121, O45, O103, and O145 serogroups in fecal samples prior to loadout. In SILN samples, STEC and serogroups O26, O121, and O45 were detected, though treatment did not impact prevalence ($P>0.05$). The prevalence of STEC in SILNs from control, BIOPLUS® 2B, and Probicon L28 market hogs was 4.5%, 2.6%, and 2.2%, respectively. When compared to the control, SILN STEC reductions achieved by BIOPLUS® 2B ($P=0.401$) and Probicon L28 ($P=0.289$) were not significant. Throughout the study, *E. coli* O157:H7 was not detected in feces or SILNs, and prevalence of the O111 serogroup was very low (0.6%) in fecal samples. All prevalence values are based upon results from the BAX® System.

Conclusions

The addition of BIOPLUS® 2B and Probicon L28 to the finishing diets of market hogs did not impact the prevalence of STEC or the O26, O121, O45, O103, and O145 serogroups in fecal samples collected from market hogs. A numerical reduction in SILN STEC prevalence was observed for BIOPLUS® 2B and Probicon L28, in comparison to the control, although these reductions were not significant. A future study with larger sample numbers and higher STEC prevalence may be more effective at determining if the addition of BIOPLUS® 2B or Probicon L28 to market hog finishing diets can significantly reduce STEC in SILNs. Isolating, confirming, and characterizing colonies from fecal and SILN samples identified as positive (according to the BAX® System results) for STEC or the O26, O111, O121, O45, O103, and O145 serogroups is an ongoing portion of this study.