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Exploring the Use of Probicon L28 and BIOPLUS 2B as Direct-Fed Microbials to Reduce Salmonella and Shiga Toxin-Producing Escherichia coli in Market Pigs

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Exploring the Use of Probicon L28 and BIOPLUS2B as Direct-Fed Microbials to Reduce *Salmonella* and Shiga Toxin-Producing *Escherichia coli* in Market Pigs[1]

Jimeng Bai, Macie E. Reeb, Mike D. Tokach, Jordan T. Gebhardt,[2] Jason C. Woodworth, Robert D. Goodband, Joel M. DeRouchey, Jessie Vipham, Qing Kang,[3] John W. Schmidt,[4] Dayna M. Brichta-Harhay,[3] Joseph M. Bosilevac,[3] Morgan Miller,[5] and Sara E. Gragg

Summary

Pigs are hosts for *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) and these pathogens can commonly be isolated from the pig farm environment. Pigs can carry pathogens to the abattoir and contaminate pork products, posing a risk to public health. Identifying an intervention that effectively reduces pathogens in commercial pigs before harvest is imperative. Due to the need for effective pre-harvest interventions in the pig industry, the objective of this study was to investigate BIOPLUS 2B (*Bacillus licheniformis* and *Bacillus subtilis*) and Probicon L28 (*Lactobacillus salivarius* L28) as pre-harvest interventions to reduce *Salmonella* and STEC in commercial growing-finishing pigs. Two groups of pigs (group 1, N = 294; group 2, N = 356, initial body weight = 106.6 lb) were fed a standard corn-soybean meal (SBM) finishing diet according to the following treatments: Probicon L28 supplementation through water lines at 1.0×10^6 CFU/head/day (Probicon); BIOPLUS 2B supplemented at 3.0×10^9 CFU/head/day (BIOPLUS 2B); and a control with no added probiotic (Control). With each group of pigs, 12 pens were used per treatment (N = 24 total), for a total of 36 pens per group (N = 72 pens total). Each group was sampled upon arrival/baseline, midway through the grow-finish phase/6 weeks post-placement, and prior to loadout/13 weeks post-placement to collect fecal samples (4 pigs/pen), boot covers (2/pen), and ropes (1/pen). Market pigs were followed to the abattoir and superficial inguinal lymph nodes (SILNs) were collected. All samples were analyzed for STEC (*stx,*

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eae genes, and O157:H7, and O26, O111, O121 O45, O103, and O145 serogroups) and *Salmonella* using the BAX System (real-time polymerase chain reaction). Overall, *Salmonella* and O111 prevalence was very low for all sample types, and *Escherichia coli* O157:H7 was not detected in any samples throughout the study. When compared to the control, there was no evidence ($P > 0.05$) that BIOPLUS 2B and Probicon L28 impacted the prevalence of STEC (*stx* and *eae* genes) or serogroups O26, O121, O45, O103, and O145 in feces, boot covers, ropes, and SILNs of market pigs.

Introduction

Salmonella is a foodborne pathogen that is commonly associated with swine and pork products, including the lymph nodes of market pigs. Shiga toxin-producing *Escherichia coli* (STEC) is also present in swine and pork products, although it is often considered to be more associated with ruminant animals than market pigs. *Salmonella* and STEC contamination can be found on the farm and transferred from the pig to the carcass during slaughter, which may result in contaminated pork products. A large body of literature reports on the use of probiotics as pre-harvest interventions, particularly in cattle. When fed to animals pre-harvest, probiotics are generally considered safe and many studies suggest they may be efficacious at reducing foodborne pathogens in the live animal. *Lactobacillus salivarius* L28 (Probicon L28) is a newly identified type of *Lactobacillus*, while BIOPLUS 2B—a probiotic product that is commonly used in the pig industry—consists of *Bacillus licheniformis* and *Bacillus subtilis*. The objective of this study was to evaluate the supplementation of BIOPLUS 2B and Probicon L28 as pre-harvest interventions to reduce *Salmonella* and STEC in market pigs. The companion report[6] provides corresponding growth performance data.

Procedures

Experimental design

The study was conducted at the Kansas State University Swine Research and Teaching Facility and the protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (#4485). Each pen was fitted with a 2-hole dry single-sided feeder (Farmweld, Teutopolis, IL) and a 1-cup waterer to provide feed and water.

Each pig had 0.72 square meters (7.8 square feet) of floor space and was located on a fully slatted concrete floor with a 1.21-meter (4 feet) pit underneath for liquid storage. Pens were organized by treatment in the barn and barriers made of hard plastic dividers were erected between pens of different treatments to eliminate nose to nose contact between animals of different treatments. Only pigs in pens of the same treatment were allowed nose to nose contact between pens. Two groups of market pigs were included in this project (Group 1, N = 294 pigs; Group 2, N = 356, initial body weight across both groups = 106.6 lb). A total of 36 pens were used for each group of commercial pigs, 12 pens were used for each treatment, with 8 to 10 pigs per pen. Each pen was assigned to one of the diets.

[6] M.E. Reeb, J. Bai, M.D. Tokach, J.T. Gebhardt, J.C. Woodworth, R.D. Goodband, J.M. DeRouchey, J. Vipham, Q. Kang, J.W. Schmidt, D.M. Brichta-Harhay, M. Miller, and S.E. Gragg. 2023. Determining the Impact of Probicon L28 and BioPlus 2B on Finishing Pig Growth Performance and Carcass Characteristics. Kansas Agricultural Experiment Station Research Reports: Vol. 9: Iss. 7.

Each pen was sampled at each of three different occasions throughout the feeding period: 1) upon arrival (baseline measurement); 2) midway (approximately 6 weeks on trial); and 3) before loadout (approximately 13 weeks on trial). During each sampling period, the following samples were collected from each pen: feces from four arbitrarily selected pigs in each pen, two boot covers (worn by person who collected fecal samples); and one rope (hung on each pen during sampling). On the last day of the trial, the final pen weights and individual weight of the pigs were obtained, and the pigs were tattooed with unique identification by pen to facilitate identification of each treatment, then were shipped to a USDA-FSIS inspected processing facility for collection of superficial inguinal lymph nodes. During shipment, the pigs were loaded onto a truck according to treatments, and one treatment was placed on each truck. In lairage, the pigs were maintained as separate treatments. All samples were analyzed for STEC (*stx, eae* genes and, O157:H7, and O26, O111, O121 O45, O103, and O145 serogroups) and *Salmonella* using the BAX System (details below).

Treatment diets

The pen was considered the experimental unit and was assigned to one of three treatments: 1) a standard corn-SBM finishing diet with Probicon L28 supplemented through water lines with a target concentration of 1.0×10^6 CFU/head/day using a water medicator system (Model D14MZ10; Dosatron International, Clearwater, FL), which diluted the stock solution to water ratio approximately 1:75 to achieve a target concentration of 1.0×10^6 CFU/head/day; 2) 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 3) a standard corn-SBM diet serving as the control. Diets were fed in 3 phases and all diets were manufactured at a commercial feed mill (Hubbard Feeds, Beloit, KS). A robot was used as a feeding system (FeedPro, Feedlogic Corp, Wilmar, MN) and it recorded the amount of feed provided for this project.

The Probicon L28 stock solution was prepared daily as described in detail in the companion report by Reeb et al., 2023.[6]

Pre-harvest sample collection

Pig feces

Feces were collected from four arbitrarily selected pigs in each pen. Using a gloved hand, one finger was inserted into the anus and gently rotated in a circular motion to manually stimulate defecation. Gloves were changed after each fecal sample was collected to ensure no cross contamination. Each sample was placed into a 24 oz (710 mL) Whirl-Pak bag (Nasco, Fort Atkinson, WI), and bags were stored in a cooler with ice packs for further processing.

Boot covers

During fecal sample collection, one person entered the pen wearing a double layer of boot covers, where the internal boot cover provided a barrier between the external boot cover and the boot. Once the pig feces were collected, the outer layer boot covers were placed into separate 55 oz (1.63 L) Whirl-Pak bags (2 boot cover samples per pen), and the inner layer boot covers were discarded. Gloves were replaced after each boot cover sample collection to ensure no cross contamination. Sample bags were stored in a cooler with ice packs for further processing.

Ropes

Twisted cotton blend 1⁄2-inch ropes (Koch, Tractor Supply Company, Manhattan, KS) were cut to a 24-inch (61 cm) length and tied to the fence of each pen to ensure that pigs could chew the rope. Each rope sample was collected at each sampling point and placed into a 55 oz (1.63 L) Whirl-Pak bag after thirty minutes. Gloves were changed after each rope collection to ensure no cross contamination. After collection, each sample was placed in a cooler with ice packs until further processing.

Harvest sample collection

Lymph nodes

Pigs were shipped on trucks to a commercial pork processor where superficial inguinal lymph nodes (SILNs) were collected during harvest. The SILNs (Group 1, N = 262; Group 2, N = 314) were placed in zip top bags and SILNs from different treatments were placed in separate bags. After collection, each sample bag was placed in a cooler with ice packs until further processing.

Pre-harvest sample processing

Pig feces

Pig fecal samples (N = 144 per sampling period) were weighed to ensure a 10 g sample, then mixed with 90 mL 107.6°F pre-warmed (42°C) BAX MP media (Hygiena, Camarillo, CA) containing Quant solution (QS: Hygiena, Camarillo, CA) at a concentration of 0.5 mL/L to prepare a feces homogenate (FH). Ten mL of 107.6°F (42°C) pre-warmed BAX MP media with QS at a concentration of 0.5 mL/L was mixed with 10 mL FH into sterile Whirl-Pak bags to make 20 mL of diluted FH samples (dFH). All 20 mL dFHs were incubated at 107.6°F (42°C) for 24 h.

Boot covers

Boot cover samples (N = 72 per sampling period) were stomached by a Smasher (bioMérieux, Marcy-l'Étoile, France) with 100 mL of buffered peptone water (BPW; Hygiena, Camarillo, CA) and homogenized for 1 min to prepare a boot cover homogenate (BCH). Thirty mL of BCH was transferred into a Whirl-Pak bag and mixed with thirty mL 107.6°F pre-warmed (42°C) BAX MP containing 1 mL/L QS as diluted BCH (dBCH). All dBCHs were incubated at 107.6°F (42°C) for 24 h.

Ropes

Rope samples were hand massaged for 1 min with 300 mL of prewarmed BAX MP with QS at a concentration of 1 mL/L. Rope homogenate (RH) was incubated at 107.6°F (42°C) for 24 h.

Harvest sample processing

Lymph nodes

Superficial inguinal lymph nodes were trimmed to remove all surrounding fat and fascia, weighed, and then dipped in boiling water for 3–5 sec. The SILNs were placed into Whirl-Pak filter bags (24 oz) and smashed by a rubber mallet. Twenty mL 107.6°F pre-warmed (42°C) BAX MP media was added to SILNs of 0 to 3 grams and 80 mL of 107.6°F pre-warmed (42°C) BAX MP was added to SILNs greater than 3 grams and samples were homogenized by a smasher for 1 min, resulting in a lymph node homogenate (LNH). All LNH samples were incubated at 107.6°F (42°C) for 24 h.

STEC and Salmonella detection

After incubation, 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] were used to detect STEC. More specifically, all samples were screened for the *stx* and *eae* genes. Samples that screened positive for both genes were then subjected to Panel 1 and Panel 2 to determine the presence of O groups in each positive sample. Therefore, all serogroup data presented were obtained from samples that were positive for the *stx* and *eae* genes. The BAX System Real-Time *Salmonella* Assay was utilized to detect *Salmonella*. Immunomagnetic separation (IMS) was used for all pig feces, boot cover, ropes, and SILN samples positive for *Salmonella* based on BAX System results. The IMS methods are described below.

STEC isolation and characterization

Immunomagnetic separation was used for STEC positive SILN samples based on BAX System results. Automatic IMS (KingFisher; Thermo Scientific, Waltham, MA) was conducted according to manufacturer's recommendations using IMS bead (Romer Lab, Newark, DE) pools that matched the O serogroup results provided by the BAX System. Following IMS, 5 uL was removed from the IMS tube for 1:50 dilution by PBS-Tween (PBS-T; Thermo Fisher; Waltham, MA), 50 µL diluted beads were spread plated on ChromAgar STEC (ChromAgar, Paris, France), 50 µL undiluted beads were plated on ChromAgar STEC, and a 10 µL loop was used to streak undiluted beads on ChromAgar STEC. If samples were positive for STEC (*stx and eae*) but not an O group based on the BAX System results, they were streaked to ChromAgar STEC directly from the sample. All plates were incubated at 98.6°F (37°C) for 24 hours. Pink colonies were picked, with at least four colonies on each plate, and two additional colonies were picked when using more than one O group bead in a pool for IMS. All pink colonies were transferred to 96-well blocks filled with 1 mL of TSA (BD Biosciences; Franklin Lakes, NJ) in each well and incubated at 98.6°F (37°C) for 24 h. The 96-well blocks were refrigerated and sent to the United States Meat Animal Research Center (USMARC; Clay Center, NE) for serotyping and further characterization.

The PCR Master Mix 500 rxns were used for determining O serogroups of O26, 45, 103, 111, 121 and 145. The PCR Mix was combined 1375 µL 10 × Buffer II with 15 mM $MgCl_2$, 510 µL of O- Group I Primer Mix, 250 µL dNTPs, and 9.75 mL ddH_2O. The number of samples were determined, 19.6 µL of the PCR Mix was mixed with 0.4 µL of Taq polymerase for each sample. The EHEC- 59 PCR program was run with a series of temperature cycles. After PCR, the resulting products were analyzed on a 1.7% agarose gel through electrophoresis, and the O serogroups were interpreted based on the specific product sizes.

Statistical analysis

Statistical analyses were performed separately for each sample type and prevalence type (STEC and individual serogroups). All tests were conducted at the 0.05 significance level. Comparisons between two levels of a fixed effect were carried out using two-sided tests. Statistical analyses were performed using the Statistical Analysis Software (SAS 9.4; Cary, NC).

For feces and boot cover samples, prevalence data were organized at the pen level as a binomial outcome. The binomial prevalence data at 6 and 12 weeks were analyzed using

the logit linear mixed model. Fixed effects of the model included replication, treatment, time, and treatment-by-time interaction. The pen-level prevalence rate at baseline served as a numeric covariate. The random effect was pen. Distributions of test statistics were approximated by chi-square distributions. The *P*-values were obtained via the Wald test. Fixed effects were evaluated in terms of model-based estimates of prevalence rates, their 95% Wald confidence intervals, and odds ratios. Statistical analyses were carried out using the SAS GLIMMIX procedure.

For rope samples, the prevalence data at 6 and 12 weeks were analyzed separately, at each sampling time, using the exact conditional logistic regression approach because of limited sample size (i.e. one sample per pen at each sampling time). The baseline prevalence status (consisting of three levels: either negative, positive, or missing) served as a categorical covariate.

For SILN samples, the prevalence data were analyzed using the exact conditional logistic regression approach because of low overall STEC prevalence rate (18/576 ≈ 3.1%). In both analyses, the replication served as the stratifying variable for the conditional inference and the fixed effect was treatment. The estimated prevalence rates of treatments were not available in conditional inference. Therefore, the raw prevalence rates and their 95% Pearson-Clopper confidence intervals of each treatment were reported. Treatments were compared via exact odds ratios and exact score-test *P*-values. Statistical analyses were performed using the SAS LOGISTIC and FREQ procedures.

Results and Discussion

STEC and serogroup prevalence

Statistical analyses were performed separately for each sample type and prevalence type (STEC [*stx* and *eae* genes] and individual serogroups). Therefore, data are presented according to prevalence for STEC and each individual serogroup.

Pig feces

The main effect of treatment did not significantly ($P > 0.05$) impact the prevalence of STEC (*stx* and *eae* genes) or serogroups O26, O121, O45, O103, and O145 in fecal samples of market pigs at loadout. The main effect of time was significant for STEC, O26, O45, and O145 ($P < 0.05$), with prevalence decreasing throughout the feeding period. A treatment by time interaction was observed for STEC ($P = 0.001$) and O121 ($P = 0.003$; Table 1). In comparison to the control diet, significant differences only occurred at the 6-week sampling point. A statistical difference was observed between BIOPLUS 2B and the control at 6 weeks, with the prevalence of STEC, O26, and O121 higher ($P < 0.05$) in BIOPLUS 2B than the control. Similarly, the O26 and O121 prevalence was higher ($P < 0.05$) at 6 weeks in pigs fed the Probicon diet in comparison to the control diet. By the 13-week sampling point (prior to loadout), STEC, O26, and O121 prevalence in pigs treated with BIOPLUS 2B and Probicon L28 were not different than the control ($P > 0.05$). The prevalence of O111 was sporadically detected and prevalence was very low throughout the trials, and no fecal samples were positive for *E. coli* O157:H7.

Boot covers

The main effect of treatment did not significantly ($P > 0.05$) impact the prevalence of STEC (*stx* and *eae* genes) or serogroups O26, O121, O45, O103, and O145 in boot

covers worn in the pens of market pigs. The main effect of time was significant for STEC and all serogroups ($P < 0.05$), except for O103 ($P = 0.514$). In general, prevalence decreased throughout the feeding period. A treatment by time interaction was not observed for STEC (*stx* and *eae* genes) or serogroups O26, O121, O45, O103 and O145. The prevalence of O111 was very low throughout the trials, and no boot covers were positive for *E. coli* O157:H7. For six pens, boot cover samples collected at weeks 6 and 13 in group 1 and at baseline in group 2 were contaminated and, therefore, excluded from data analysis and statistical modeling.

Ropes

The prevalence of STEC, O26, O121, O45, O103, and O145 serogroups from ropes hung in pens of pigs fed diets supplemented with BIOPLUS 2B and Probicon L28 did not vary at weeks 6 or 13 ($P > 0.05$) in comparison to ropes from pens of pigs fed the control finishing diet. *Escherichia coli* O157:H7 and O111 were not detected throughout the trial in rope samples.

Lymph nodes

From the 576 SILNs collected, the BAX System detected STEC (*stx* and *eae* genes) and serogroups O26, O121, and O145. In comparison to the control, finishing diets supplemented with BIOPLUS 2B and Probicon L28 did not impact prevalence ($P > 0.05$). Although not significant ($P > 0.05$), a numerical reduction in STEC and O121 prevalence was observed for BIOPLUS 2B and Probicon L28, in comparison to the control group (Table 2).

Salmonella prevalence

Salmonella prevalence was very low throughout this study. Across the two groups of pigs, *Salmonella* prevalence ranged from 0.7% to 2.8% and *Salmonella* prevalence in boot covers ranged from 0% to 2.8%. The *Salmonella* prevalence of rope samples ranged from 0.0% to 10% across both pig groups. *Salmonella* prevalence of SILNs in group 1 and group 2 market pigs was 0.8% and 0%, respectively.

STEC characterization

A total of 150 isolates were recovered and further characterized, of which 56 were found to be serogroup O121 possessing the *stx2e* subtype, and 51 of which were isolated from lymph nodes. Of these 51 isolates, 39 were recovered from lymph node samples collected from Group 1 pigs fed the control treatment. Twelve isolates were recovered from a Group 2 SILN collected from a pig fed the BIOPLUS 2B diet (Table 3).

While significant differences were observed during the 6-week (midway) sampling period, the results showed that supplementing market pig finishing diets with BIOPLUS 2B or Probicon L28 during the finishing period had no effect ($P > 0.05$) on the prevalence of STEC or *E. coli* O26, O121, O45, O103, and O145 in pig feces, boot covers, ropes, and lymph node samples of commercial pigs at loadout or at the abattoir when compared with pigs fed a control finishing diet. *Escherichia coli* O121 with *stx2e* only (lacking *eae*) was isolated from SILN samples and is usually associated with edema disease in pigs but has little effect on human health. The prevalence of *Salmonella* throughout the experiment was very low, which limited power and the ability to detect a treatment effect, should one exist. Larger sample sizes and/or enrolling animals with a higher prevalence of pathogens, particularly *Salmonella,* would improve power and

the ability to determine if BIOPLUS 2B or Probicon L28 would impact the pathogen prevalence in market pigs when supplemented into finishing diets.

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Table 1. Treatment effects in the analyses of Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* O26, and *E. coli* O121 prevalence from the feces of pigs fed control, BIOPLUS 2B, and Probicon diets for 6 and 13 weeks during the finishing period[1,2]

Sample type	Serogroup	Time, weeks	Treatment	Positive rate (95% conf. int.)	Odds ratio to (*P*-value)
Pig feces	STEC	6	Control	22.9% (14.5%,34.1%)	--
			BIOPLUS 2B	50.0% (37.2%,62.7%)	3.37 (<.001)
			Probicon	36.2% (24.2%,50.2%)	1.91 (0.110)
		13	Control	10.0% (5.3%,18.2%)	--
			BIOPLUS 2B	5.1% (2.2%,11.2%)	0.48 (0.168)
			Probicon	19.0% (11.1%,30.7%)	2.12 (0.113)
	O26	6	Control	14.3% (8.2%,23.7%)	--
			BIOPLUS 2B	43.4% (32.0%,55.6%)	4.59 (<.001)
			Probicon	30.1% (20.0%,42.5%)	2.57 (0.025)
		13	Control	3.5% (1.4%,8.9%)	--
			BIOPLUS 2B	3.3% (1.2%,9.0%)	0.95 (0.939)
			Probicon	8.8% (4.4%,16.9%)	2.65 (0.117)
	O121	6	Control	1.3% (0.3%,5.4%)	--
			BIOPLUS 2B	9.3% (4.6%,17.8%)	7.96 (0.011)
			Probicon	10.3% (5.2%,19.4%)	8.94 (0.008)
		13	Control	6.2% (2.7%,13.4%)	--
			BIOPLUS 2B	1.6% (0.4%,6.5%)	0.24 (0.087)
			Probicon	2.9% (1.0%,8.4%)	0.46 (0.253)

[1] Probicon: A standard corn-soybean meal finishing diet with Probicon L28 supplemented through water lines at a target concentration of 1.0×10^6 CFU/head/day using a water medicator system. BIOPLUS 2B: A standard corn-soybean meal finishing diet supplemented with BIOPLUS 2B (5.0×10^8 CFU/pound of feed; ~3.0×10^9 CFU/head/day). Control: A standard corn-soybean meal diet.

[2] Samples considered positive for STEC and serogroups O26 and O121 were screened as positive for possessing the *stx* and *eae* genes.

Table 2. Treatment effects in the analyses of Shiga toxin-producing *Escherichia coli* (STEC) prevalence status for lymph node samples from pigs fed control, BIOPLUS 2B and Probicon diets during the finishing period[1,2]

Sample type	Serogroup	Treatment	Positive rate (95% Conf. int.)	Odds ratio to (*P*-value)
Lymph node	STEC	Control	4.5% (2.1%,8.5%)	--
		BIOPLUS 2B	2.6% (0.8%,5.9%)	0.53 (0.401)
		Probicon	2.2% (0.6%,5.4%)	0.45 (0.289)
	O26	Control	0.0% (0.0%,1.8%)	--
		BIOPLUS 2B	0.0% (0.0%,1.9%)	NA
		Probicon	0.5% (0.0%,3.0%)	1.02 (0.494)
	O121	Control	3.0% (1.1%,6.5%)	--
		BIOPLUS 2B	1.0% (0.1%,3.7%)	0.32 (0.276)
		Probicon	0.5% (0.0%,3.0%)	0.17 (0.134)
	O145	Control	1.0% (0.1%,3.6%)	--
		BIOPLUS 2B	1.0% (0.1%,3.7%)	1.01 (1.000)
		Probicon	1.6% (0.3%,4.7%)	1.60 (0.945)

[1] Probicon: A standard corn-soybean meal finishing diet with Probicon L28 supplemented through water lines at a target concentration of 1.0×10^6 CFU/head/day using a water medicator system. BIOPLUS 2B: A standard corn-soybean meal finishing diet supplemented with BIOPLUS 2B (5.0×10^8 CFU/pound of feed; ~3.0×10^9 CFU/head/day). Control: A standard corn-soybean meal diet.

[2] Samples considered positive for STEC and serogroups O26, O121, and O145 were screened as positive for possessing the *stx* and *eae* genes.

Table 3. Shiga toxin-producing *Escherichia coli* isolated from lymph nodes of Group 1 and Group 2 pigs fed control, BIOPLUS 2B and Probicon diets during the finishing period[1]

	Treatment	Lymph node isolates[2]
Group 1	Control	39
Group 2	Bioplus 2B	12

[1] Probicon: A standard corn-soybean meal finishing diet with Probicon L28 supplemented through water lines at a target concentration of 1.0×10^6 CFU/head/day using a water medicator system. BIOPLUS 2B: A standard corn-soybean meal finishing diet supplemented with BIOPLUS 2B (5.0×10^8 CFU/pound of feed; ~3.0×10^9 CFU/head/day). Control: A standard corn-soybean meal diet.

[2] All isolates were confirmed as serogroup O121 possessing the *stx2e* subtype, but lacking *eae*.