TITLE: Diets including two different Direct-Fed Microbials neither Reduce *Salmonella* or Shiga Toxin-Producing *Escherichia coli* in Market Pigs nor impact Finishing Pig Growth Performance and Carcass Characteristics

RUNNING TITLE: Probiotics Pathogens Performance Market Pigs

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ABSTRACT

Pigs are hosts for *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC). Identifying an intervention that effectively reduces pathogens in commercial pigs before harvest is imperative. This study evaluated the probiotics BIOPLUS® 2B and Probicon L28 as direct-fed microbials to 1) reduce *Salmonella* and STEC in the lymph nodes and feces of commercial growing-finishing pigs, and 2) improve growth performance and carcass characteristics. Market pigs were fed a standard corn-soybean meal finishing diet according to the following treatments: Probicon L28 at 1.0×10^6 CFU/head/day (Probicon); BIOPLUS® 2B at 3.0×10^9 CFU/head/day (BIOPLUS® 2B); and a control (Control). Fecal samples, boot covers, and ropes were collected upon arrival (baseline), 6wk post-placement, and prior to loadout/13 wk post-placement. Superficial inguinal lymph nodes (SILNs) were collected at the abattoir. All samples were analyzed for *Salmonella* and STEC. Pen (~21-day intervals) and individual weights were used to assess growth performance. Carcasses were evaluated by hot carcass weight, loin depth, backfat, and percentage lean. Overall *Salmonella* and STEC O111 prevalence were very low in all sample types, and *Escherichia coli* O157:H7 was not detected. There was no evidence ($P>0.05$) that BIOPLUS® 2B and Probicon L28 impacted the prevalence of STEC (*stx* and *eae* genes) or STEC serogroups O26, O121, O45, O103 and O145 in feces, boot covers, ropes, and SILNs of market pigs. No evidence of difference ($P>0.10$) between treatment was observed for overall average daily gain, average daily feed intake, gain:feed ratio, or any carcass traits. These data suggest that the probiotics used in this study had no impact on growth or carcass characteristics of finishing pigs, or prevalence of *Salmonella* and STEC in fecal samples or SILNs of market pigs.

KEYWORDS: *Escherichia coli*, *Salmonella*, probiotics, performance, market pigs, interventions

According to Centers for Disease Control and Prevention estimates, foodborne diseases cause 48 million people to become ill, 128,000 people to be hospitalized, and 3,000 people to die each year (Centers for Disease Control and Prevention, 2022). *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* are considered major causes of foodborne disease outbreaks in the United States (Marshall et al., 2020). Non-typhoidal *Salmonella* is responsible for the most hospitalizations and deaths from foodborne illnesses (Centers for Disease Control and Prevention, 2022). An estimated 265,000 STEC infections occur annually in the United States, of which 36% are caused by STEC O157 and 64% by non-O157 STEC (Centers for Disease Control and Prevention, 2014). While the CDC can present estimates, the actual number of infections is likely to be higher because not all STEC infections are diagnosed, either because many infected people do not seek health care or because many patients who do seek health care do not provide stool samples for testing (Centers for Disease Control and Prevention, 2014).

Numerous reviewed studies have reported that STEC is present in pork products, although often at much lower levels than in ruminant products, and producers and consumers still need to be concerned about possible STEC contamination in pork products (Haque et al., 2022; Svoboda & Cutter, 2012). Similar to *Salmonella*, STEC contamination originates at the farm and can be transferred from the pig to the carcass during slaughter, thereby contaminating pork and commodities (Colello et al., 2016). Conversely, *Salmonella* and STEC not only cause a health threat to humans but can also make pigs sick and lead to economic losses. Infection with *Salmonella* can present in pigs in two ways, clinical or subclinical (Neumann et al., 2020). Clinical signs most commonly present as diarrhea or sepsis, however, the majority of pig herds are asymptomatic/subclinical carriers of *Salmonella* (Neumann et al., 2020). *Salmonella* burdens

the swine industry economic losses due to increased pharmaceutical use, reduced weight gain, reduced feed to gain ratio, prolonged time to market, and loss of productivity due to changes in carcass weight (Ainslie-Garcia et al., 2018). In pigs, Stx2e-producing STEC poses serious economic challenges as it may cause edema disease (Mesonero-Escuredo et al., 2021). (Remfry et al., 2021). Edema disease affects healthy, rapidly growing nursery pigs, with varied clinical signs that may include marked edema of the gastric submucosa and mesocolon, sudden death in pigs, and paralysis (Fairbrother, 2023).

Probiotics are generally considered safe and are often recognized for the many benefits they confer to humans and animals, including protection against pathogen infection and regulation of gut microbiota (Shi et al., 2020). Probiotics containing *Lactobacillus* are effective at reducing foodborne pathogens in feces and hides, and their potential to replace antibiotics has increased their use in the livestock industry (Ayala et al., 2017). Probiotics have been reported to improve the effectiveness of the intestinal barrier by producing antibacterial compounds such as bacteriocins and organic acids, competing with pathogenic bacteria for epithelial receptors and nutrients in the gastrointestinal tract, and producing enzymes and vitamins (Ayala et al., 2017). *Lactobacillus salivarius* L28 (Probicon) was isolated recently from ground beef (Flach et al., 2022). Preliminary data suggested that *Lactobacillus salivarius* L28 could be a viable alternative to antibiotic use since it had similar effects on weight gain, performance, and carcass traits in beef cattle while also controlling *E. coli* and *Salmonella* in cattle feces (Flach et al., 2022). *Bacillus subtilis* and *Bacillus licheniformis* are considered probiotics because of their ability to produce enzymes and secondary metabolites that inhibit the growth of pathogenic microorganisms (Romo-Barrera et al., 2021). The combination of *Bacillus licheniformis* and *Bacillus subtilis* has been shown to have anti-inflammatory and antioxidant effects, which inhibit

the pathogenic bacteria adhesion (Palkovicsné Pézsa et al., 2022). BIOPLUS® 2B is a probiotic product, consisting of *Bacillus licheniformis* and *Bacillus subtilis*, and is commonly used in the pig industry (Chr Hansen, 2023). The objective of this study was to evaluate the supplementation of BIOPLUS® 2B and Probicon L28 as a pre-harvest intervention to 1) reduce *Salmonella* and Shiga toxin-producing *Escherichia coli* in market pig feces and lymph nodes, and 2) determine if the supplements impact performance or carcass traits.

MATERIALS AND METHODS

All procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol #4485) and the Institutional Biosafety Committee. Due to the need to apply dietary and water treatments, study personnel were not all blinded to study treatments; however, extensive efforts were made to blind most study personnel. For example, pens were labeled by letters (A, B, C) and colors (red, blue, yellow) to minimize bias and ensure measurements were as objective as possible.

Experimental design. The study was conducted at the Kansas State University Swine Research and Teaching Facility. 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. The facility is totally enclosed and environmentally regulated. Each pig had 0.72 square meters of floor space and was located on a fully slatted concrete floor with a 1.21-meter pit underneath for liquid storage. Pens were organized by treatment in the barn (Figures 1 and 2) 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 pens of the same treatment were allowed nose to nose contact between pens. Two groups of pigs (group 1 N=294 and group 2 N=356, initial BW=48.4 kg) were enrolled in this study. For each group, a total of 36 pens were used (N=72

pens total), with 12 pens per treatment (N=24 pens total), with approximately 10 pigs per pen. Pigs were randomly assigned to pen via a completely randomized design, and pens were assigned to one of three treatments (details below).

Treatment diets. The pen was considered the experimental unit and was assigned to one of three treatments. The control pigs were fed a standard corn-soybean meal (SBM) diet. The two experimental diets included probiotic supplementation with a standard corn-SBM diet. For the first experimental treatment, a standard corn-SBM diet was supplemented with BIOPLUS® 2B at a concentration of 5.0×10^8 CFU/pound (454 kg) of feed or approximately 3.0×10^9 CFU/head/day, which is equivalent to 1.1×10^6 CFU/g of feed. For the second experimental treatment, pigs were fed a standard corn-SBM diet and Probicon L28 was supplemented through water lines with a target concentration of 1.0×10^6 CFU/head/day. The probiotic was administered 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.

Daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record the amount of feed provided for individual pens. Dietary treatments were fed in meal form in three body weight phases from approximately 36 to 63, 82 to 91, and 91 to 132 kg (Table 1). All diets were manufactured at a commercial feed mill (Hubbard Feeds; Beloit, KS).

Probicon L28 stock solution preparation and concentration. The Probicon L28 stock solution was prepared daily and consisted of tap water, activated Probicon L28 and electrolyte powder, pH of 6.5. Briefly, 2 g of Probicon Calf (NexGen Innovations, LLC, Lubbock, TX) were combined in 10 mL of tryptic soy broth (TSB; BD BioSciences; Franklin Lakes, NJ) and

activated for 10 hours at 37ºC and stored at 4ºC until use (up to 72 hours). Each day, a fresh Probicon L28 stock solution was prepared in a clean 5-gallon bucket by combining 17 kg of tap water with 17 g of electrolyte powder (DuMor Multi-Species Electrolytes Supplement, distributed by Tractor Supply Company, Brentwood, TN) and one tube of activated Probicon L28. Prior to adding the Probicon L28, the stock solution pH was adjusted to ~6.5 using distilled white vinegar (Great Value, Bentonville, AR). The average estimated Probicon intake was calculated based on 1) the daily pig water intake record, and 2) laboratory estimates for stock solution concentration, group 1 consuming an average of 6.7×10^4 CFUs per pig per day and group 2 consuming 1.2×10^5 CFUs per pig per day. More specifically, stock solution intakes were recorded by subtracting the weight of the remaining solution when a new solution was prepared, by the total stock solution weight from the previous day. Daily water intakes for all treatments were recorded via a water meter. Thus, the dilution percentage was calculated by the stock solution intake divided by the daily water intake. As the dilution percentage was tracked over the course of the two groups of pigs and minor adjustments to the dilution of the water medication system (Model D14MZ10; Dosatron International, Clearwater, FL) were done to reach target supplementation rate.

Growth, performance and carcass characteristics. Pen weights and feed disappearance were recorded approximately every 21 d throughout the study. On the last day of the trial, final pen and individual weights were obtained. Data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed:gain (F/G). Prior to loadout, pigs were tattooed with a treatment and pen identification number and transported to a U.S. Department of Agriculture-inspected abattoir for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Percentage lean was calculated

from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Pre-harvest sample collection. 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). From each pen, feces, boot covers, and ropes were collected during each sampling period. All samples were analyzed for STEC (*stx, eae* genes and, O157:H7, and O26, O111, O121 O45, O103, O145 serogroups) and *Salmonella* using the BAX® System. Immunomagnetic separation (IMS) was used to isolate *Salmonella* and STEC for further characterization.

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 (Five Rock SafeTrack-HD®, QC Supply, Dallas, TX), 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 ½-inch ropes (Koch Industries Inc., Minneapolis, MN) were cut to a 24-inch length and tied to the fence of each pen to ensure that pigs could chew the rope for a minimum of 15-30 minutes. Rope samples were collected at each sampling point and placed into a 55oz 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.

Transportation, lairage, and harvest sample collection. On the last day of the trial, the final pen weight 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 shipped to a USDA-FSIS inspected processing facility for collection of superficial inguinal lymph nodes, MicroTally™, and trim samples. During shipment, the pigs were loaded onto a truck according to treatments, and one treatment was placed on each truck (one truck per treatment). In lairage, the pigs were maintained as separate treatments, with 1-2 lairage pens per treatment.

Lymph nodes. Superficial inguinal lymph nodes (SILNs) were collected at the abattoir. 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, with multiple SILNs from a single treatment in one bag. After collection, each sample bag was placed in a cooler with ice packs until further processing.

Trim and MicroTally™. When the head was split, head and cheek meat trim from each diet was held in plastic totes, with a separate tote for each treatment. Using a MicroTally™ cloth (Fremonta; San Jose, CA), each tote was manually sampled for 30 seconds using one side of the cloth, followed by another 30 seconds using the second side of the cloth. Gloves were changed between each treatment. After collection, MicroTally™ cloths were returned to their original plastic sample bag and stored in a cooler with packs until further processing. Each treatment of

trim samples was randomly sampled using clean forceps, and enough trim was collected to fill one 55oz. (1.63 L) Whirl-Pak homogenizer blender filter bag. All sample bags were placed in a cooler with ice packs until further processing.

Ropes in Lairage. At the abattoir, the pigs were divided into lairage pens according to their treatment. Ropes with 3 to 4 inches length were hung by the processor in each lairage pen for a minimum of 45 minutes and provided for each treatment. After collection, rope samples were stored 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 was mixed with 90 mL 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 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 42˚C for 24 hours.

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 minute to prepare a boot cover homogenate (BCH). The entire boot cover was used to prepare this BCH. Thirty mL of BCH was transferred into a Whirl-Pak bag and mixed with thirty mL pre-warmed (42˚C) BAX MP containing 1 mL/L QS as diluted BCH (dBCH). All dBCHs were incubated at 42˚C for 24 hours.

Ropes. Rope samples were hand massaged for 1 minute with 300 mL of prewarmed BAX MP with QS at a concentration of 1 mL/L. Rope homogenate (RH) was incubated at 42˚C for 24 hours.

Harvest sample processing

Lymph Nodes. Superficial inguinal lymph nodes were trimmed to remove all surrounding fat and fascia, weighed and then boiled for 3 to 5 seconds. The SILNs were placed into Whirl-Pak filter bags (24 oz) and smashed by a rubber mallet. Twenty mL pre-warmed (42˚C) BAX MP media was added to SILNs of 0 to 3 grams and 80 mL pre-warmed (42˚C) BAX MP was added to SILNs greater than 3 grams and homogenized by a smasher for 1 minute, resulting in a lymph node homogenate (LNH). All LNH samples were incubated at 42˚C for 24 hours.

Trim. Head and cheek meat samples were processed using the N60 method, where 60 small pieces totaling 375 g were homogenized with 1,500 mL of pre-warmed (42˚C) BAX MP. All trim homogenate (TH) samples were incubated at 42˚C for 24 hours.

MicroTally™. Each MicroTally™ was weighed, homogenized by a smasher with 200 mL pre-warmed (42˚C) BAX MP for 1 minute, and incubated at 42˚C for 24 hours.

Ropes. Rope samples were added to 300 mL of prewarmed BAX MP with QS at a concentration of 1 mL/L and homogenized by hand for 1 minute. Rope homogenate (RH) was incubated at 42˚C for 24 hours.

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] and BAX® System Real-Time *Salmonella* Assay were utilized to detect STEC and *Salmonella* for all samples. More specifically, lysates were prepared according to the BAX® System Real-Time *Salmonella* Assay package insert, and this lysate was used for SalQuant®, *Salmonella* detection, and the BAX® System Real-Time STEC Screening Suite. All samples were screened for the *stx* and *eae* genes, and 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. Immunomagnetic separation (IMS) was used for all pig fecal, boot cover, rope, and SILN samples positive for *Salmonella* based on BAX® System results. The IMS methods are described below in section 4.2.9.

Salmonella *quantification.* The BAX® System Real-Time *Salmonella* Assay was utilized to quantify *Salmonella* as per manufacturer's guidelines. Briefly, at 10 hours of incubation time, pig fecal samples were removed from the incubator and lysates were prepared. For boot cover samples, lysates were prepared after 8 hours of incubation time. At 6 hours of incubation time, lysates were made for lymph nodes, trim, MircoTally™, and rope samples. These lysates were held at 4ºC until all samples were analyzed for *Salmonella* detection at 24 hours as described above. Samples identified as positive for *Salmonella* at 24 hours were then quantified using the corresponding lysate prepared previously and stored at 4ºC.

STEC isolation and characterization. Only samples that were 1) collected at the abattoir, and 2) identified as STEC-positive based on BAX® System results were subjected to isolation and characterization protocols. Automated IMS (KingFisher™; Thermo Scientific™, Waltham, MA) was conducted according to manufacturer's recommendations using IMS bead (Romer Lab, Newark, Delaware) pools specific to O serogroups identified 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 on to ChromAgar STEC the remaining IMS supernatant that the beads had been collected from. If samples were positive for STEC (*stx and eae*) but not an O

group based on BAX® System results, they were streaked for isolation to ChromAgar STEC directly from the sample using a 10 µL loop. All plates were incubated at 37˚C for 24 hours. Pink colonies were picked, with at least four colonies on each plate, and when using more than one O group bead in a pool for IMS two additional colonies were picked per IMS bead in the pool. All pink colonies were transferred to 96-well blocks filled with 1 ml TSA (BD Biosciences; Franklin Lakes, NJ) in each well and incubated at 37˚C for 24 hours. The 96-well blocks were refrigerated and sent to the United States Meat Animal Research Center (USMARC) for serotyping and further characterization.

Salmonella *isolation and characterization.* The lymph node, trim, MicroTally™, and rope samples collected during harvest that were identified as positive using the BAX® System were streaked on xylose lysine desoxycholate (XLD) agar (Remel, Thermo Fisher, Waltham, MA). When direct streaking was unable to recover a colony, automated IMS with anti-*Salmonella* beads (Fisher Scientific, St. Louis, MO) was conducted using manufacturer's recommendations where one hundred µL of bead-bacteria complex generated by IMS were transferred to 3 mL Rappaport Vassiliadis (RV; BD BioSciences; Franklin Lakes, NJ) broth, and incubated at 42˚C for 18 hours. Incubated RV tubes were streaked for isolation on XLD agar, and all XLD plates were incubated at 37˚C for 24 hours. After incubation, three well-isolated, presumptive *Salmonella* colonies (black on XLD) from each plate were re-streaked on XLD (one plate per colony), and incubated 37˚C for 24 hours. One well-isolated colony from each re-streak plate was picked and transferred to a 10 mL tube of TSB, and TSB tubes were incubated 37˚C for 24 hours. Each TSB tube was confirmed as *Salmonella* using the BAX® System Real-Time *Salmonella* Assay. One mL from each TSB tube that confirmed as *Salmonella* on the BAX®

System was frozen with a final concentration of 10% glycerol (BD BioSciences; Franklin Lakes, NJ) in microtubes in triplicate at -80°C.

Salmonella *serotyping.* *Salmonella* serotypes were determined using molecular serotyping methods (Echeita et al., 2002; Herrera-León et al., 2004; Herrera-León et al., 2007). Unresolved isolates were identified and molecular serotypes were confirmed using commercial antisera (BD Diagnostic Systems, Sparks, MD) to perform traditional slide agglutination (O-typing) and tube agglutination (flagellar H typing).

STEC serogrouping. STEC serogroups of O26, O45, O103, O111, O121, O145, and O157 were identified by multiplex PCR and serology as described previously (Bosilevac & Koohmaraie, 2011). Briefly, each STEC isolate was first examined for common serogroups (O26, O45, O103, O111, O121, O145, and O157) then if no O group was identified, serologic O grouping was performed using antisera (Cedarlane Labs, Burlington, Canada) acording the package inserts.

Growth, performance and carcass characteristics statistical analysis. Data were analyzed as a completely randomized design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.2 (2018-07-02) with pen as the experimental unit and treatment considered as a fixed effect. Group was included in the model as a random intercept. Carcass data were analyzed with the addition of pen nested within group as a random intercept to account for subsampling of multiple carcass observations within each experimental unit. All carcass characteristics (lean percentage, loin depth, backfat) included HCW in the model as a covariate.

Microbiological statistical analysis. Statistical analyses were performed separately for each sample type and prevalence type (STEC and STEC of individual serogroups as identified by the BAX® System screening). 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 rep, 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. 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 BAX® System-determined STEC prevalence rate ($18/576 \approx 3.1\%$). In both analyses, rep 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

Growth, performance, and carcass characteristics. Overall ADG, ADFI, and F/G were not significantly ($P > 0.10$) effected by treatment Pigs fed the BIOPLUS® 2B treatment had numerically greater loin depth compared to other treatments, but there were no significant pairwise differences between treatments ($P>0.05$).

STEC and serogroup prevalence. Prevalence is based upon samples identified as positive by the BAX® System for STEC **(***stx* and *eae* genes) and each *E. coli* serogroup (O26, O111, O121, O45, O103 and O145). More specifically, only samples identified as positive by the BAX® System for both the *stx* and *eae* genes were 1) considered positive for STEC, and 2) analyzed by BAX® System STEC Panel 1 and Panel 2 to determine the presence or absence of each serogroup. Therefore, a sample considered positive for a serogroup has been characterized by the BAX® System as follows: *stx*+, *eae*+, and O group+ (*e.g. stx*+, *eae*+, and O26+). For the purposes of this article, STEC will refer to samples that are *stx*+ and *eae*+, and STEC-O group (*e.g.* STEC-O26) will be used to refer to samples that satisfy the *stx*+, *eae*+, and O group+ criteria described herein.

Pig feces. The main effect of treatment did not significantly ($P>0.05$) impact the prevalence of STEC (*stx* and *eae* genes) or STEC serogroups O26, O121, O45, O103 and O145 in fecal samples of market pigs at loadout. The main effect of time was significant for STEC, STEC-O26, STEC-O45, and STEC-O145 ($P<0.05$), with prevalence decreasing throughout the feeding period. A treatment by time interaction was observed for STEC ($P=0.001$) and STEC-O121 ($P=0.003$), and nearly observed for STEC-O26 ($P=0.052$) (Table 3). In comparison to the control, 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, STEC-O26, and STEC-O121 higher (P<0.05) in BIOPLUS® 2B than the control (Table 3). Similarly, the STEC-O26 and STEC-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, STEC-O26, and STEC-O121 prevalence in pigs treated with BIOPLUS® 2B and Probicon L28 were not different than the control (P>0.05). The prevalence of STEC-O111 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 STEC 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 STEC-serogroups (P<0.05), except for STEC-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 STEC serogroups O26, O121, O45, O103 and O145. The prevalence of STEC-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 6 and 13 weeks 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, STEC-O26, STEC-O121, STEC-O45, STEC-O103, and STEC-O145 serogroups from ropes hung in pens of pigs fed diets supplemented with BIOPLUS® 2B and Probicon L28 did not vary at 6 weeks or 13 weeks (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. Ten samples at baseline in group 1, 3 samples

at 6 weeks and 6 samples at 13 weeks in group 2 were not able to be collected and thus not used for data analysis and statistical modeling.

Lymph nodes. From the 576 SILNs collected, the BAX® System detected STEC (*stx* and *eae* genes) and STEC 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 STEC-O121 prevalence was observed for BIOPLUS® 2B and Probicon L28, in comparison to the control group (Table 4).

Trim and MicroTally™. For group 1 pigs, one trim sample collected from the control treatment tested positive for STEC (*stx* and *eae* genes), and STEC serogroups O26, O121, O45, O103, and O145. One rope from the Probicon treatment was positive for STEC serogroups O26, O121 and O103. A rope from the control treatment was positive for serogroups STEC-O121, STEC-O45, and STEC O145. A rope from BIOPLUS® 2B was positive for serogroups O121 and O103. One trim sample from the BIOPLUS® 2B treatment of group 2 tested positive for STEC (*stx* and *eae* genes) and STEC serogroups O26, O121, O45, O103, and O145.

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 to 10% across both pig groups. *Salmonella* prevalence of SILNs in group 1 and group 2 market pigs was 0.8 and 0%, respectively. *Salmonella* was detected in all trim (N=6), MicroTally™ (N=6), and rope samples (N=6) collected from the abattoir.

Salmonella *quantification.* *Salmonella* was quantified in a total of thirteen samples using the SalQuant® methodology. Due to the small number of quantified samples, data are not

described according to treatment, group of pigs, or sampling date. Concentration was generally low, with five (38.5%) samples (two fecal, one trim, one MicroTally™ trim, and one boot cover) harboring *Salmonella* at a concentration below the limit of quantification [1 (0 $\log_{10}$) *Salmonella* cell per gram or mL]. Of the remaining eight samples that were quantifiable, six were associated with rope samples that ranged from 0.9 to 2.5 $\log_{10}$ *Salmonella* cells per mL (3.2 to 4.8 $\log_{10}$ *Salmonella* cells per rope). One fecal sample harbored *Salmonella* at a concentration of 0.3 $\log_{10}$ *Salmonella* cells per g (2.3 $\log_{10}$ *Salmonella* cells per sample), while 1.7 $\log_{10}$ *Salmonella* cells per g (4.3 $\log_{10}$ *Salmonella* cells per sample) was the concentration of one trim sample.

STEC and* Salmonella *isolation. Of the 18 SILNs, 3 rope, and 2 trim samples positive for STEC, automatic IMS recovered isolates from 7 samples. A total of 150 isolates presumptive for STEC were recovered, and 56 isolates were characterized as O121 with *stx2e* only (lack *eae*). Of these 56 isolates, 39 were recovered from lymph node samples collected group 1 pigs fed the control treatment. Twelve isolates were recovered from a lymph node sample and five isolates were recovered from a trim sample collected from group 2 pigs fed the BIOPLUS® 2B diet (Table 5).

Among the 2,087 samples included in this study, *Salmonella* was isolated from 28 samples. From each positive sample, up to three colonies were isolated and subjected to serotyping for a total of 78 *Salmonella* isolates (Table 6). From these 78 isolates, *Salmonella* Agona was the most frequently isolated serotype (N=23 isolates), originating from boot covers, feces, MicroTally™, ropes, and trim. Twelve isolates were identified as Senftenberg from feces, boot covers, and ropes. *Salmonella* Braenderup was identified for 9 isolates, originating from feces, boot covers, and ropes. Nine isolates were identified as monophasic Typhimurium that were recovered from trim, a rope, and MicroTally™ samples. The remaining serotypes were represented by six

isolates or less, and included Worthington, Stanley, Montevideo, Derby, Muenchen, Typhimurium, Uganda, Saintpaul, and London. The most isolates (25.6%) were recovered from lairage ropes (N=20). From samples collected at the abattoir, SILNs were the only sample type that did not produce *Salmonella* isolates.

DISCUSSION

The present study did not indicate that supplementation with BIOPLUS® 2B or Probicon L28 during the finishing period had an impact on overall ADG, ADFI, G:F, or carcass characteristics measured at the abattoir. Alexopoulos et al. (2004) administered BIOPLUS® 2B at a concentration of 1.28×10^6 CFU/g of feed to pigs from the time of weaning to 120 days of age and reported an economic benefit due to 1) a reduction in morbidity, 2) improved growth performance during the growing and finishing periods, and 3) improved carcass quality. Although the present study administered BIOPLUS® 2B at a similar concentration (1.28×10^6 CFU/g of feed), the probiotic was only fed during the finishing period, whereas Alexopoulos et al. (2004) fed BIOPLUS® 2B at the time of weaning until 120 days of age (during finishing). This difference may have resulted in differing results between Alexopoulos et al. (2004) and the results described herein.

Because Probicon L28 is a probiotic that is new to the market, data are limited in the literature. This study is the first to report on the use of Probicon L28 as a direct-fed microbial in finishing pigs; however, the use of Probicon L28 has been reported in the literature for beef cattle. Flach et al. (2022) reported that unpublished, preliminary data from their laboratory team indicate that *Lactobacillus salivarius* L28 had similar effects on weight gain, performance, and carcass traits of beef cattle when compared to the use of sub-therapeutic antibiotics, suggesting that Probicon L28 may be a viable solution for reducing the use of antibiotics in the cattle

industry. Additional data is necessary in the swine industry to fully elucidate the impact of Probicon L28 on performance and carcass parameters of finishing pigs.

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 STEC serogroups O26, O121, O45, O103 and O145 at loadout when compared with pigs fed a control finishing diet. The *Salmonella* prevalence was too low to detect a treatment effect. Although this study did not demonstrate that the use of Probicon L28 or BIOPLUS® 2B as direct feeding microorganisms in pigs reduced *Salmonella* and STEC in market pigs, there is a large body of research that suggests the use of DFMs pre-harvest can reduce pathogens in animals.

Flach et al. (2022) reported that DFMs in feedlots can reduce the prevalence of foodborne pathogens in feces and peripheral lymph nodes (PLNs) of beef cattle (Flach et al., 2022). Three treatments were used throughout this study, including control, Bovamine, and Probicon L28. Over a five-month period, fecal samples were collected and tested for *E. coli* O157:H7 and *Salmonella,* and results showed a reduction in foodborne pathogens in feces from cattle supplemented with both DFMs, and a significant reduction in the prevalence of *E. coli* O157:H7 and *Salmonella* in feces collected from pens from cattle supplemented with Probicon L28 (Flach et al., 2022).

Lewton et al. (2022) reported on the early health benefits for nursery pigs fed a DFM prepared from multiple strains of *Bacillus subtilis* (DFM). The authors used intestinal mucosa, plasma immune markers, and intestinal morphology as criteria. Pigs supplemented with the DFM increased the total plasma level of IgA by more than 20% compared with control pigs and increased the villus height of the jejunum (Lewton et al., 2022). These data suggest that nursery

pig health can benefit from supplementation with *Bacillus subtilis*. The literature generally suggests that *Bacillus* spp. probiotics are beneficial for young pig health (Liu et al., 2018; Luise et al., 2022), while efficacy of DFMs at improving pig health is somewhat variable in the published literature and may vary based upon diet (Liu et al., 2018).

In another study, pigs treated with BIOPLUS® 2B had lower morbidity and mortality than control pigs throughout the trial period, which was due to a lower incidence of post-weaning diarrhea that was mainly caused by *E. coli* (Alexopoulos et al., 2004). Alexopoulos et al. (2004) also stated that probiotics are not always beneficial to pigs, as they are not a panacea and should be used only after critical thought. Failure in one study doesn't mean probiotics don't work, rather it is important to recognize that experimental designs vary greatly, there are differences in the pathogens and animal species being tested, and variability in immunomodulator system, and farm health can also lead to significant differences in results (Alexopoulos et al., 2004). Future studies with larger sample sizes and higher prevalence of *Salmonella* and STEC may be more effective in determining whether supplementation of BIOPLUS® 2B or Probicon L28 in market pig finishing diets significantly reduces *Salmonella* and STEC in pre-harvest pigs.

In the present study, of the 23 SILN, trim, MicroTally™, and rope samples collected at the abattoir that were positive for STEC, 150 isolates were recovered from 7 samples, and 56 were characterized as O121 with *stx2e* only (lack *eae*). During an 11-year study, a total of 2,231 *E. coli* isolates from pigs were recovered, 233 of which were *stx2e* producing isolates, and 109 isolates were able to be typed by O-antisera, of which 87 were O139 serogroup. Although attention is often focused on *E. coli* O157, the serogroups that cause edema in post weaning and fattening pigs are often O8, O138, O139, and O147. *Escherichia coli* O157 might be an important serogroup from a public health perspective in humans, but in other animals, other

serogroups may also have significant health implications (Baldo et al., 2020). In another study, from 598 samples collected from 10 pig flows, a total of 178 isolates were recovered and 23 serogroups were identified , among which the three major serogroups were O8, O86 and O121 (Remfry et al., 2021). Of the 178 isolates, 26 isolates carried *stx1a* and 152 isolates carried *stx2e*. Strains isolated with *stx1a,* particularly those associated with *eae*, might cause serious human infections (Remfry et al., 2021). While the STEC subtypes recovered in this study are associated with neonatal diarrhea, post-weaning diarrhea, and edema disease, the authors concluded they were of low public health importance (Remfry et al., 2021).

There are many serotypes of *Salmonella* that are associated with pigs, but Choleraesuis and Typhimurium have been known to cause clinical illness in pigs. In addition, Agona, Derby, Heidelberg and Infantis serotypes may lead to diarrhea in pigs that can range from mild to moderate (Burrough, 2022). Of these serotypes, Heidelberg and Infantis were not recovered from pigs enrolled in this study. *Salmonella* Choleraesuis is the primary cause of most salmonellosis in pigs; however, infections in other animals and humans are rare (Burrough, 2022), and this serotype was not recovered in this study.

In 2014, the three most common serotypes of *Salmonella* isolated from pork and pork products in the European Union (EU) were *Salmonella* Typhimurium, *Salmonella* Derby, and *Salmonella* monophasic Typhimurium (Bonardi, 2017). In the present study, all three of these *Salmonella* serotypes were also identified. In 2015, there was an outbreak of *Salmonella* Typhimurium in North Carolina involving pork barbecues, which infected 280 people and resulted in one death (Clark, 2015). In 2011, outbreaks of *Salmonella* Typhimurium and *Salmonella* Derby that linked to dried pork sausages occurred in several towns in Spain, of which 18% of infected people need medical care (Arnedo-Pena et al., 2016).

CONCLUSIONS

Supplementation of BIOPLUS® 2B or Probicon L28 in finishing diets of market pigs had no effect on the prevalence of STEC or STEC serogroups 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. *Escherichia coli* O121 with *stx2e* only (*eae* negative) 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, limiting the power and ability to detect a treatment effect. Of the *Salmonella* serotypes recovered, Agona was most frequently isolated, but several serotypes associated with outbreaks in humans (pork or other products) were also identified, with the presence of Typhimurium (including monophasic Typhimurium) perhaps most notable from a public health perspective. 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 impact pathogen prevalence in market pigs when supplemented into finishing diets.

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Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of its name by USDA implies no approval to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request.

CONFLICT OF INTEREST STATEMENT

The authors have no competing or financial interests associated with publication of this article.

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FIGURES AND TABLES

Figure 1. Barn map of finishing diets assigned to pens of group 1 market pigs (8-10 pigs per pen) during the finishing period[1]. The treatment assignments varied between groups to reduce the potential effect of location with the barn.

39 BioPlus®2B	22 BioPlus®2B
38 BioPlus®2B	23 BioPlus®2B
37 BioPlus®2B	24 BioPlus®2B
36 Probicon	25 Probicon
35 Probicon	26 Probicon
34 Probicon	27 Probicon
33 Control	28 Control
32 Control	29 Control
31 Control	30 Control

19 Control	2 Control
18 Control	3 Control
17 Control	4 Control
16 BioPlus®2B	5 BioPlus®2B
15 BioPlus®2B	6 BioPlus®2B
14 BioPlus®2B	7 BioPlus®2B
13 Probicon	8 Probicon
12 Probicon	9 Probicon
11 Probicon	10 Probicon

[1] Probicon: A standard corn-soybean meal (SBM) 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-SBM 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-SBM diet.

Figure 2. Barn map of finishing diets assigned to pens of group 1 market pigs (8-10 pigs per pen) during the finishing period[1]. The treatment assignments varied between groups to reduce the potential effect of location with the barn.

39 Control	22 Control
38 Control	23 Control
37 Control	24 Control
36 Probicon	25 Probicon
35 Probicon	26 Probicon
34 Probicon	27 Probicon
33 BioPlus®2B	28 BioPlus®2B
32 BioPlus®2B	29 BioPlus®2B
31 BioPlus®2B	30 BioPlus®2B

19 BioPlus®2B	2 BioPlus®2B
18 BioPlus®2B	3 BioPlus®2B
17 BioPlus®2B	4 BioPlus®2B
16 Control	5 Control
15 Control	6 Control
14 Control	7 Control
13 Probicon	8 Probicon
12 Probicon	9 Probicon
11 Probicon	10 Probicon

[1] Probicon: A standard corn-soybean meal (SBM) 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-SBM 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-SBM diet.

Table 1. Diet composition (as-fed basis)[1]

Item, %	Phase 1	Phase 2	Phase 3
Corn	70.91	77.66	86.29
Soybean meal	26.35	19.89	11.54
Calcium carbonate	1.01	0.95	0.88
Monocalcium phosphate	0.62	0.46	0.33
Sodium chloride	0.35	0.35	0.35
L-Lys-HCl	0.29	0.30	0.30
DL-Met	0.06	0.05	0.01
L-Thr	0.11	0.10	0.09
L-Trp	0.01	0.02	0.03
Vitamin premix with phytase[2]	0.15	0.13	0.10
Trace mineral premix	0.15	0.13	0.10
Direct fed microbial[3]	+/-	+/-	+/-
Total, %	100	100	100
Calculated analysis			
Standard ileal digestible (SID) AA, %			
Lys	1.05	0.90	0.70
Ile:Lys	63	62	60
Leu:Lys	136	142	155
Met:Lys	31	31	30
Met and Cys:Lys	56	57	58
Thr:Lys	64	64	65
Trp:Lys	19.2	19.0	19.3
Val:Lys	70	70	70
His:Lys	42	43	44
Total Lys, %	1.18	1.02	0.79
NE, kcal/lb	1,116	1,136	1,161
SID Lys:NE, g/Mcal	4.27	3.59	2.73
CP, %	18.8	16.3	13.0
Ca, %	0.66	0.57	0.49
P, %	0.50	0.44	0.38
STTD P, %	0.38	0.32	0.27
Ca:P	1.30	1.30	1.30

[1]Phase 1 was fed from approximately 80 to 140 lb, Phase 2 from approximately 140 to 200 lb, and Phase 3 from approximately 200 to 290 lb.
[2]Vitamin premix contained phytase (Ronozyme HiPhos, DSM Nutritional Products, Parsippany, NJ) to provide 750, 624, and 498 FYT/kg diet and provided an estimated release of 0.11%, 0.10%, and 0.09% STTD P for phases 1, 2, 3, respectively.
[3]Direct fed microbial was added to the respective treatment (BIOPLUS® 2B, CHR Hansen Inc, Milwaukee, WI) at 0.05% of the diet at the expense of corn.

Table 2. Effects of BIOPLUS® 2B or Probicon L28 as direct-fed microbials on finishing pig performance and carcass characteristics[1]

Item	Control	BIOPLUS® 2B	Probicon	SEM	*P* =
BW, kg					
d 0	48.3	48.5	48.3	2.63	0.948
d 83	130.9	132.1	131.4	6.42	0.628
Overall (d 0 to 83)					
ADG, kg	1.00	1.01	1.01	0.040	0.705
ADFI, kg	2.65	2.65	2.65	0.235	0.975
G:F, g/kg	379	383	382	19.0	0.376
Carcass characteristics[2]					
HCW, kg	97.2	97.9	97.2	4.61	0.648
Carcass yield, %	74.1	74.2	74.3	0.43	0.825
Lean, %	55.4	55.6	55.3	0.41	0.182
Loin depth, cm	6.62	6.73	6.66	0.112	0.070
Back fat depth, cm	1.42	1.42	1.46	0.065	0.133

[1] A total of 650 pigs (initial BW = 48.4 kg) were used in a 83-d finisher trial with approximately 10 pigs per pen and 24 pens per treatment. Pigs were allotted to treatment in a completely randomized design. BIOPLUS® 2B (CHR Hansen Inc, Milwaukee, WI) was added at 0.05% of the diet at the expense of corn. Probicon L28 (Benebios, Inc, Mishawaka, IN) was supplemented through water lines using a water medicator system at a target concentration of 1.0×10^6 CFU/head/day.
[2]In the analysis for backfat depth, percentage lean, and loin depth, HCW was used as a covariate in the model.

Table 3. 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]. Samples considered positive for STEC and serogroups O26 and O121 were screened as positive for possessing the *stx* and *eae* genes.

Sample Type	Serogroup	Time	Treatment	Pos. Rate (95% Conf. Int.)	Odds Ratio to (P-value)
Pig Feces	STEC	6 wk	Control	22.9% (14.5%,34.1%)	--
			BIOPLUS® 2B	50.0% (37.2%,62.7%)	3.37 (<0.001)
			Probicon	36.2% (24.2%,50.2%)	1.91 (0.110)
		13 wk	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 wk	Control	14.3% (8.2%,23.7%)	--
			BIOPLUS® 2B	43.4% (32.0%,55.6%)	4.59 (<0.001)
			Probicon	30.1% (20.0%,42.5%)	2.57 (0.025)
		13 wk	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 wk	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 wk	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 (SBM) 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-SBM 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-SBM diet.

Table 4. Treatment effects in the analyses of STEC prevalence status for lymph node samples from pigs fed control, BIOPLUS® 2B and Probicon diets during the finishing period[1]. Samples considered positive for STEC and serogroups O26, O121, and O145 were screened as positive for possessing the *stx* and *eae* genes.

Sample Type	Serogroup	Treatment	Pos. Rate (95% Conf. Int.)	Odds Ratio to (P-value)
SILN	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 (SBM) 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-SBM 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-SBM diet.

Table 5. 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]	Trim
Group 1	Control	39	--
Group 2	BIOPLUS® 2B	12	5

[1] Probicon: A standard corn-soybean meal (SBM) 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-SBM 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-SBM diet.

[2] All isolates were confirmed as serogroup O121 possessing the *stx2e* subtype

Table 6. *Salmonella* serotypes isolated from pig feces, boot covers, ropes, lymph nodes, MicroTally™, and trim from group 1 and group 2 market pigs fed control, BIOPLUS® 2B and Probicon diets during the finishing period[1].

	Sampling Point[2] and Sample Type													
	Baseline			6 Weeks Post-Placement			13 Weeks Post-Placement			Abattoir				
Salmonella Serotype	Feces	Boot Covers	Ropes	Feces	Boot Covers	Ropes	Feces	Boot Covers	Ropes	Lymph Nodes	MicroTally	Trim	Lairage Rope	Total
Agona	3	6							3		4	2	5	23
Senftenberg					3	3	3						3	12
Braenderup					3	3							3	9
Monophasic Typhimurium											5	3	1	9
Worthington					6									6
Stanley									5					5
Montevideo								3						3
Derby													3	3
Muenchen											1	1		2
Typhimurium													2	2
Uganda												1	1	2
Saintpaul													1	1
London													1	1
TOTAL	3	6	0	0	12	6	3	3	8	0	10	7	20	78

[1] Probicon: A standard corn-soybean meal (SBM) 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-SBM 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-SBM diet.

[2] Baseline: upon arrival to the finishing barn; 6 weeks: approximately midway on trial; 13 weeks: before loadout.

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