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A Dose-Response Investigation of a Micronized Porous Ceramic Particle to Improve the Health and Performance of Post-weaned Pigs Infected With *Salmonella enterica* Serotype Typhimurium

*Emily M. Davis[1], Kayla P. Wallace[2], Michael J. Cruz Penn[2], Amy L. Petry[3], Rand Broadway[4], Nicole C. Burdick Sanchez[4], Jeffery A. Carroll[4] and Michael A. Ballou[1]**

[1] Department of Veterinary Sciences, Texas Tech University, Lubbock, TX, United States, [2] Texas Tech University School of Veterinary Medicine, Amarillo, TX, United States, [3] Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, United States, [4] Livestock Issues Research Unit, Agricultural Research Service, United States Department of Agriculture (USDA), Lubbock, TX, United States

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***Correspondence:**
Michael A. Ballou
michael.ballou@ttu.edu

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The objective was to investigate the effects of supplementing increasing concentrations of PowerGuard (PG), a micronized ceramic particle, to weaned pigs on health and performance following a *Salmonella* enterica serotype Typhimurium infection. Forty barrows were transported to the USDA facility in Liberty, TX, USA. Pigs were randomly assigned to one of five treatments ($n = 8$): (1) uninfected control (CON), no *Salmonella* typhimurium (ST) and no PG treatment; (2) infected control (ST), infected with ST but no PG treatment; (3) PG0.05, infected with ST and supplemented with 0.05% PG; (4) PG0.25, infected with ST and supplemented with 0.25% PG; and (5) PG0.50, infected with ST and supplemented with 0.5% PG. All pigs were enrolled at 21.5 ± 1.33 days of age and did not differ in initial BW (1.98 ± 0.09 kg). Pigs were anesthetized to insert temperature recording devices into the abdominal cavity. Pigs were offered feed and water *ad libitum*. Pigs in ST, PG0.05, PG0.25, and PG0.50 were infected orally with 1.75×10^7 colony-forming units of *Salmonella* typhimurium on day 7. Pig body weights and peripheral blood samples were collected on days 0, 7, 10, 14, and 21. Pigs were harvested on day 21 and ileum and liver samples were collected for histopathological analyses. There was no treatment difference for final BW ($P \geq 0.201$). There was a tendency ($P = 0.087$) for a treatment difference in the fecal score; ST and PG0.50 had more loose fecal scores than CON and PG0.25. There was a treatment × time interaction for intraperitoneal temperature ($P < 0.0001$); PG0.05, PG0.25, and PG0.50 had attenuated febrile responses during the acute post-infection period compared with ST. There was a treatment × time interaction for total leukocyte counts ($P = 0.007$); PG treatments reduced leukocytosis post-infection compared with ST. Supplementing PG0.25 improved many health and performance variables when pigs were infected with *Salmonella* Typhimurium. Furthermore, supplementing PG0.05 attenuated the febrile response and many hematological variables. However, supplementing PG0.5 did not

improve many aspects of health or performance. Therefore, supplementing PowerGuard between 0.05 and 0.25% of the diet may play a role in protecting weaned pigs from disease caused by *Salmonella*.

Keywords: biotoxin binder, clay, infection, montmorillonite, nutraceutical

INTRODUCTION

Many *Salmonella enterica spp.* are zoonotic pathogens that can have negative health and productive impacts on pigs and may also increase human exposure to food-borne pathogens. When *S. enterica* serotype Typhimurium colonizes the intestinal tract of a host, bacteria can invade local lymph nodes throughout the gastrointestinal tract and colonize there (Rostagno et al., 2011; Broadway et al., 2015). The invasion of internal organs, shedding *via* feces, and carcass contamination from digesta, all create potential reservoirs for disruption in pre-harvest food safety in the swine industry. Human infections of *S. enterica* serotype typhimurium ranked 3rd of total reported cases, accounting for 9.8% of total culture-confirmed *Salmonella* cases in the United States. From herd monitoring data collected in part by the USDA, pigs accounted for 13% of clinical *Salmonella* typhimurium cases in submitted animal samples (Center for Disease Control, 2013, 2016). The European Food Safety Authority (EFSA) approximates that pork is responsible for 10–20% of total *Salmonella* infections in humans in the European Union (EFSA, 2011). Furthermore, it was previously reported that finishing pigs can carry high concentrations of *Salmonella* typhimurium, between 10^3 and 10^5 CFU/g, in the gastrointestinal tract and associated lymph nodes for up to 4 weeks post-infection, while showing no clinical signs of disease (Rostagno et al., 2011). Along with the contamination potential, there are health and production losses that *S. enterica* infections can cause in pigs prior to harvest. In the EU, the combined cost of human and pig production losses totals 600 million Euros (FCC, 2010); more specifically, Danish reports state *Salmonella* causes roughly a 3 kg BW loss per infected pig (Lo Fo Wong and Hald, 2000). In the swine industry, *S. enterica* treatment commonly involves antibiotics. However, with the growing concern of antibiotic resistance and the increase in restrictions on antibiotic usage, the shift to nutraceutical strategies is increasing. Currently, clay is being used in the agriculture industry as a mycotoxin binder. Previously, weanling pigs fed mycotoxin contaminated diets with clay supplementation reported that the pigs fed clay either increased or maintained performance when compared to pigs not supplemented with clay (Schell et al., 1994; Thieu et al., 2008; Jiang et al., 2010; Wang et al., 2012). With a high adsorption capacity for mycotoxins, clays have been under recent investigation for the ability to adsorb to gram-negative bacteria, including *S. enterica* and *Escherichia coli* (Song et al., 2012; Pardo et al., 2020).

The nutraceutical utilized in the current study is a thermally processed montmorillonite-based micronized ceramic particle. When montmorillonite clay is thermally processed, there is the removal of the outer- and inter-layer water, which changes the physical nature of the clay to become a ceramic particle (Bala et al., 2000). Once this ceramic is created, the particle cannot be rehydrated back into a lump of clay. Along with the heat processing, there are changes in physical porosity as well. Thus, the myriad of potential adsorptive mechanisms may allow for ceramic particles to better adsorb and mediate the effects of gram-negative pathogenic bacteria such as *Salmonella* typhimurium. The pathogen load in pigs prior to and during harvest is the ultimate determinant of contamination along the farm-to-consumption pathway and determines the infection potential in the post-harvest and consumption stage (EFSA, 2011).

Therefore, the objective of the current study was to investigate the effects of supplementing different levels of a commercial ceramic particle to weaned pigs during a *Salmonella* typhimurium infection on performance and health.

MATERIALS AND METHODS

Preliminary Bacteria Adsorbent Analyses and Electron Micrograph

Salmonella enterica serotypes Dublin and Typhimurium were utilized in a preliminary study to determine the capacity of multiple adsorbents *in vitro*. An overnight culture from each strain was created by inoculation of 20 ml of sterile tryptic soy broth and incubation at 37°C. The overnight culture was quantified by spread plate serial dilutions on tryptic soy agar plates. Dilutions with 30–150 colony forming units (CFU) were counted to determine the concentration of each isolate (CFU/ml). Approximate working concentrations of each serotype were performed in sterile phosphate-buffered saline (PBS) (2×10^6 CFU/ml). PowerGuard (PG; MB Nutritional Sciences, Lubbock, TX, USA), activated carbon (Sigma Aldrich, St. Louis, MO, USA), bentonite #1, and bentonite #2 were all diluted to 5 mg/ml concentrations in sterile PBS. Equal volumes of working bacterial solution and binder solutions were mixed into 50 ml conical tubes, along with negative control samples mixing an equal volume of each bacterial solution and sterile PBS. All cultures were performed in triplicate, and each tube was rocked for 2 h at 37°C. Then, each culture, including negative controls, was filtered through a series of sterile Whatman filter paper. First, a 25-micron paper (Whatman Grade 54, Whatman plc, Little Chalfont, Buckinghamshire, United Kingdom) followed by an 8-micron paper (Whatman Grade 2) to remove most of the adsorbent particles but to still allow un-adsorbed *Salmonella spp.* to flow through the filter paper into the filtrate. The final filtrate was clear; however, this methodology may underestimate the adsorptive capacities due to some adsorbent particle sizes being smaller than 8 microns, leading to some smaller adsorbent particles remaining in the filtrate. Each filtrate sample was serially

diluted in sterile PBS and spread plated on tryptic soy agar. Plates were incubated overnight at 37°C and plates with CFU counts of 30–150 were counted. Data were reported as the $\log_{10}$ reduction from the control to the adsorbent solution for each isolate.

For imaging purposes utilizing scanning electron microscopy, samples were prepared by the addition of 1.75×10^9 CFU/ml of *Salmonella* typhimurium in 10 ml of deionized water with 25 mg of PowerGuard. The samples were incubated at 37°C for 2 h before being fixed. The samples were then chemically fixed with 2% glutaraldehyde for 1 h, then washed with deionized water three times while centrifuging between each wash. The solutions were then quickly frozen in a dry ice and ethanol bath for 10 min followed by freezing overnight. The dried samples were mounted onto sample mounts with double-sided carbon tape and coated with a thin layer of Iridium to provide conductivity. Scanning electron microscopy (SEM) images were taken by Zeiss crossbeam 540 at 5 kiloelectron volts (kV) using secondary electrospray ionization detector (Zeiss Meditec Inc., Dublin, CA, USA). Samples were observed at the Texas Tech University Microscopy Core Laboratory by SEM with the assistance of Dr. Zhao.

Animals and Treatments

All procedures in this study were ap-proved by the USDA-ARS, Livestock Issues Research Unit's Institutional Animal Care and Use Committee (IACUC protocol #LIRU-2120S). The study was conducted in May 2021. Forty crossbred newly weaned barrows (L280 × Camborough, PIC, Inc., Hendersonville, TN, USA) were weighed (1.98 ± 0.09 kg) and transported 9 km from the Texas Tech Swine Unit to the swine barn at the Livestock Issues Research Unit. Pigs were transported mid-day when the temperature was 27°C. Pigs were randomly assigned to one of five treatments ($n = 8$): (1) Uninfected Control (CON), no *Salmonella* typhimurium (ST) administered and no treatment in the diet; (2) Infected Control (ST), infected with ST on day 7 but no treatment in the diet; (3) PG0.05, infected with ST on day 7 with 0.05% PG of the diet; (4) PG0.25, infected with ST on day 7 and 0.25% PG of the diet; and (5) PG0.5, infected with ST on day 7 and 0.5% PG of the diet. PowerGuard is a thermally processed ceramic particle that is micronized to a median particle size of 40 μm, with 90% of the particles being <100 μm. The barn was maintained at 32.2°C for days 0 and 1. The environmental temperature was then decreased by 0.56°C each day. The final barn temperature of 23.8°C was reached on day 16 and maintained for the duration of the study. Pigs were housed in individual stainless-steel pens (1.2 × 0.6 m) equipped with stainless steel automatic feeders and nipple waterers. All pigs had *ad libitum* access to feed and in order to allow *ad libitum* access to the feed, the automatic feeders had the gate open 7.5 cm, which likely allowed for pigs to waste some feed. Therefore, feed intake data and feed efficiency data are reported as feed disappearance. All pigs were enrolled on the same day, 21.5 ± 1.33 days of age, and immediately upon arrival to the facility were individually anesthetized briefly to insert temperature measuring loggers (Star-Offi DST micro-T; Metermall USA, Marysville, Ohio) into their abdominal cavity. This methodology is previously described (Burdick Sanchez et al., 2017). Peripheral blood (10 ml) was drawn in EDTA vacutainer tubes while each pig was anesthetized *via* jugular venipuncture. The entire process was completed in <10 min. Pigs had *ad libitum* access to feed and water. Diets were formulated to meet or exceed the NRC (2012) nutrient recommendations (NRC, 2012). They received the experimental diets over two dietary phases from day 0 to 6 (P1) and day 7 to 21 (P2), respectively. Samples of each diet were collected at each feeding and composited by period before being analyzed for dry matter, ash, crude protein, and ether extract by a commercial lab using wet chemistry methods (ServiTech, Amarillo, TX, USA). Formulated ingredients and diet nutrient compositions for both P1 and P2 are shown in **Table 1**.

Salmonella Typhimurium Infection

Pigs in treatments ST, PG0.05, PG0.25, and PG0.5 were all infected on day 7 with 1.75×10^7 CFU of *Salmonella* typhimurium that was resistant to nalidixic acid and in the mid-logarithmic phase of growth ATCC 14028, which was originally isolated from cardiac tissue in chickens. The infection and identification methods were adapted from Broadway et al. (2015). To summarize, an individual colony from a streak plate was placed into trypticase soy broth with nalidixic acid (50 mg/L) and incubated at 37°C agitating at 200 rpm overnight. Then, 100 μl of the overnight culture was added to 20 ml of fresh trypticase soy broth with nalidixic acid (50 mg/L) incubated at 37°C agitating at 200 rpm until reaching an optical density of 0.8 at 450 nm. This was determined previously to be the mid-logarithmic phase of growth. This broth was diluted by the addition of 1 ml of the bacterial broth with 9 ml of sterile saline for a final concentration of 1.75×10^7 CFU per the 10 ml. Sodium bicarbonate was also added to buffer the bacteria at 1 g/10 ml. This buffered bacterial solution was then administered by oral gavage utilizing a 10 ml syringe with an attached 1 mm internal diameter silicone tube, 6 mm in length, administered behind the tongue slowly as the pig swallowed within 10 min of creating the working bacteria solution. Uninfected control pigs (CON) were administered 10 ml of sterile saline with 1 g/10 ml of sodium bicarbonate prior to any handling of infectious doses or pigs in any other treatment. To estimate the infection dose, the working bacteria solution was serially diluted on trypticase soy agar with nalidixic acid at 50 mg/L and incubated overnight at 37°C. Plates were counted with colony counts between 30 and 150, and the infection dose was determined to be 1.75×10^7 CFU.

Observations and Sample Collections

Individual pig body weights and peripheral blood samples were collected on days 0, 7, 10, 14, and 21. Peripheral blood (10 ml) was collected by jugular venipuncture *via* a 20-gauge needle 1.5 inches in length in EDTA vacutainer tubes while the pig was restrained in a v-trough. Complete blood count analysis was performed on the samples using an IDEXX analyzer with the swine-specific algorithm (ProCyte Hematology Analyzer, IDEXX, Westbrook, ME, USA). Fecal swabs for bacteria prevalence were collected following Broadway et al. (2015). Two fecal swabs were collected from fresh feces from each crate beginning on day 7 prior to *Salmonella* typhimurium

TABLE 1 | The formulated diets fed to weanling pigs from day 21 of life through day 42.

	P1[a]				P2[b]			
	Control	**PG0.05**	**PG0.25**	**PG0.5**	**Control**	**PG0.05**	**PG0.25**	**PG0.5**
Ingredient, %								
Corn	44.9	44.9	44.7	44.4	55.8	55.7	55.5	55.3
Soybean meal	25.8	25.8	25.8	25.8	28.0	28.0	28.0	28.0
Whey powder	15.1	15.1	15.1	15.1	7.50	7.50	7.50	7.50
Porcine plasma	4.0	4.0	4.0	4.0	3.48	3.48	3.48	3.48
Fish meal	5.0	5.0	5.0	5.0	0	0	0	0
Soybean oil	2.0	2.0	2.0	2.0	1.50	1.50	1.50	1.50
Dicalcium phosphate	0.75	0.75	0.75	0.75	1.35	1.35	1.35	1.35
Calcium carbonate	0.59	0.59	0.59	0.59	0.45	0.45	0.45	0.45
Salt	0.5	0.5	0.5	0.5	0.50	0.50	0.50	0.50
Vitamin premix[c]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Trace mineral premix[d]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Zinc oxide	0.38	0.38	0.38	0.38	0.18	0.18	0.18	0.18
Lysine	0.28	0.28	0.28	0.28	0.50	0.50	0.50	0.50
DL-Methionine	0.18	0.18	0.18	0.18	0.25	0.25	0.25	0.25
PowerGuard®	0.00	0.05	0.25	0.50	0.00	0.05	0.25	0.50
Nutrient composition, as fed								
Analyzed nutrients								
Dry matter, %	89.8	90.0	90.2	90.2	88.3	88.7	88.7	88.8
Ash, %	6.1	6.6	6.4	6.7	5.1	5.4	5.1	5.7
Crude protein, %	24.1	22.6	24.0	23.1	20.3	21.4	20.6	21.3
Ether extract, %	4.1	4.1	4.0	4.0	3.2	3.3	3.3	3.1
Calculated nutrients[e]								
Total Lys, %	1.55	1.55	1.55	1.55	1.47	1.47	1.47	1.47
SID Lys, %	1.46	1.46	1.46	1.46	1.40	1.40	1.40	1.40
SID TSAA:Lys	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
SID Thr:Lys	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
SID Trp:Lys	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
NDF, %	5.15	5.15	5.15	5.15	6.65	6.65	6.65	6.65
NDF, %	5.15	5.15	5.15	5.15	6.65	6.65	6.65	6.65
Ca, %	0.84	0.84	0.84	0.84	0.76	0.76	0.76	0.76
STTD P, %	0.42	0.42	0.42	0.42	0.39	0.39	0.39	0.39
ME, mcal/kg	3.39	3.39	3.39	3.39	3.38	3.38	3.38	3.38
NE, mcal/kg	2.40	2.40	2.40	2.40	2.42	2.42	2.42	2.42
ZnO, ppm	3,000	3,000	3,000	3,000	1,500	1,500	1,500	1,500

[a] *P1 was a nursery diet fed from day 0 through day 6 of the study.*

[b] *P2 was a nursery-weanling diet fed from day 7 through day 21 of the study.*

[c] *The vitamin premix provided per kg of complete diet: 7,250 IU vitamin A, 850 IU vitamin D3, 50 IU vitamin E, 3.6 mg vitamin K, 13.2 mg riboflavin, 67.2 mg niacin, 32.4 mg pantothenic acid, and 60 μg vitamin B12.*

[d] *The trace mineral premix provided per kg of complete diet: 160 ppm Fe as $FeSO_4$, 160 ppm Zn as $ZnSO_4$, 9 ppm Mn as $MnSO_4$, 12 ppm Cu as $CuSO_4$, 0.3 ppm I as $C_2H_{10}I_2N_2$ or KIO_3, and 0.3 ppm Se as Na_2SeO_3.*

[e] *SID, standard ileal digestible; TSAA, total sulfur amino acids (Methionine and Cysteine); STTD, standardized total tract digestible; ME, metabolizable energy; NE, net energy.*

administration. As described by Broadway et al. (2015), one swab was placed directly into Rappaport-Vassiliadis (RV) broth (Thermo Fischer Scientific, Waltham, MA, USA) and the other swab was placed directly into Difco Tetrathionate (TT) broth (Thermo Fischer Scientific, Waltham, MA, USA). Fresh fecal swabs were collected every morning from day 7 through day 21 by two trained individuals only swabbing fresh feces. If no fresh feces were present, a fecal swab was taken directly from the rectum for that sample day. The fecal swabs in the RV broth were incubated overnight at 42°C, and the TT broth swabs were incubated overnight at 37°C. All samples were then streak plated on Xylose Lysine Deoxycholate Agar (XLD) (Thermo Fischer Scientific, Waltham, MA, USA). The plates were then incubated overnight at 37°C to determine if there was *Salmonella* colony growth *via* examination for the presence or absence of black colonies. Enrichment samples were recorded as either positive or negative. At harvest, a section of the ileum, as well as associated mesenteric lymph nodes, were collected,

rinsed, and immediately dipped into boiling water for no more than 5 s to remove potential external contamination from the harvest process for later bacterial quantification and enrichment. These samples were then stomached in a 1:10 dilution of sterile 1X PBS and sampled three subsequent times. Two swabs were collected from each tissue sample and grown overnight in both TT and RV enrichment broth and subsequently streak plated on differential XLD agar as stated previously in the fecal swabs. These samples provided enrichment data on only the presence of ST in the tissue samples and not quantification. The third sample from these stomached tissues was serially plated for *Salmonella* typhimurium quantification purposes on XLD agar as well.

Histology

On day 21, pigs were humanely euthanized. Tissue samples were collected from both the ileum and liver, washed with sterile saline to remove blood and digesta, then placed directly into 10% buffered formalin for histological analyses after hematoxylin and eosin staining. Slides were created at the Anatomical Pathology Lab at the Texas Tech University Health Sciences Center (Texas Tech University Health Science Center, Lubbock, TX, 79415). Slides were analyzed with an anatomical histopathologist at the Texas Tech School of Veterinary Medicine and scanned *via* an Olympus slide scanner for imaging and count purposes (Olympus Life Sciences, Center Valley, PA, 18034). The method of scoring histology slides is as follows. Inflammation scoring was on a 0 to 3, scale with 0 = normal tissue; 1 = mild inflammation; 2 = moderate inflammation; and 3 = marked inflammation. Villi blunting: 0 = long, slender villi; 1 = 75% of normal height; 2 = 50% of normal height; and 3 = 25% of normal height. Villi epithelium score: 0 = single layer of the columnar epithelium; 1 = degeneration, vacuolation, or separation of focal areas of the superficial epithelium; 2 = more marked changes and some focal loss of epithelium; and 3 = widespread ulceration of the epithelium surface. Lacteal dilation score: 0 = central lacteal represents approximately 25% of the width of the villi; 1 = represents approximately 50% of the villi width; 2 = 75% of the villi width; and 3 = dilated to 100% of the villi width. Goblet cell count in the crypt and intraepithelial lymphocyte counts are the number of cells counted per 50 epithelial cells. Lamina propria counts were the number of each leukocyte (lymphocytes, neutrophils, and eosinophils) observed between crypts in an x40 field. Connective tissue (CT) prominence scores ranged from 0 to 3, with a score of 0 = no prominent CT, 1 = slightly prominent CT, not diffuse; 2 = more prominent CT, throughout the sample, and 3 = all CT present is severe and prominent. Lymphocyte foci scores ranged from 0 to 3, with 0 = no lymphocyte aggregates present; 1 = few lymphocyte aggregates present; 2 = multiple lymphocyte aggregates present; and 3 = severe and diffuse lymphocyte aggregates present throughout the sample. Lymphocyte foci area was measured in μm and conducted on up to 5 foci/samples and averaged per sample. The neutrophil scores ranged from 0 to 3, with 0 = no neutrophils present in lymphocyte aggregates, 1 = <5 neutrophils within lymphocyte aggregates; 2 = between 5 and 10 neutrophils within the lymphocyte aggregates; and 3 = more than 10 neutrophils within the lymphocyte aggregates.

Statistical Analyses

Preliminary *in vitro* adsorption data were analyzed using the GLM procedure of SAS with adsorbent, *Salmonella enterica* serotype, and their interaction as the fixed effects. Continuous, repeatedly measured data were analyzed using the Mixed procedure in SAS (SAS 9.4, Cary, NC, USA). The model included fixed effects of treatment, time, and treatment × time. Initial body weight was included in the model as a covariate and retained in the model if it was significant. The subject of the repeated statement was pig nested within treatment. All covariance and variance structures were analyzed, and the most appropriate model was selected based on the smallest Bayesian information criterion. All continuous, non-repeated data were analyzed using a restricted maximum-likelihood ANOVA with treatment included as the fixed effect using the MIXED procedure of SAS. Count data were analyzed using the GLIMMIX procedure of SAS with treatment as the fixed effect and fit with a Poisson distribution using the log link function. Differences of $P \leq 0.05$ were considered significant and a tendency was reported when $0.05 < P \leq 0.1$. Treatment × time interactions found to be significant were further evaluated using a Duncan adjustment to control for familywise error. All pairwise comparisons at each significant time point were reported.

RESULTS

Bacteria Adsorption Log Reduction and Electron Micrograph

Measures of the capacity for different adsorbents to reduce bacteria concentrations reported as log base 10 reductions, in the final filtrate are reported in **Figure 1** for *Salmonella enterica* serotypes Dublin and Typhimurium. There was no adsorbent treatment × *Salmonella enterica* serotype interaction ($P = 0.696$); however, there was an adsorbent treatment difference for $\log_{10}$ reduction of both *Salmonella enterica* serotypes ($P < 0.0001$; **Figure 1**). Both PowerGuard and activated carbon had a greater $\log_{10}$ reduction of both *Salmonella enterica* serotypes when compared to two bentonite clays. The reported electron micrograph shows *Salmonella* typhimurium, highlighted in yellow, adsorbed to the surface of PowerGuard (**Figure 2**).

Performance

Measures of performance are reported in **Table 2**. There was no difference among treatments for initial body weight or final body weight ($P \geq 0.201$). There was a treatment × time interaction for feed disappearance ($P = 0.028$; **Figure 3**). There was no treatment or treatment × time interaction for ADG ($P = 0.312$). There was a tendency for a treatment × time interaction for feed disappearance to gain ($P = 0.059$; **Figure 4**); where on days 11-14 PG0.25 had decreased feed disappearance to gain when compared to both PG0.5 and CON treatments.

Hematology

Hematological counts are reported in **Table 3**. There was a treatment difference for red blood cell count ($P = 0.03$), where the CON treatment had increased red blood cell counts when

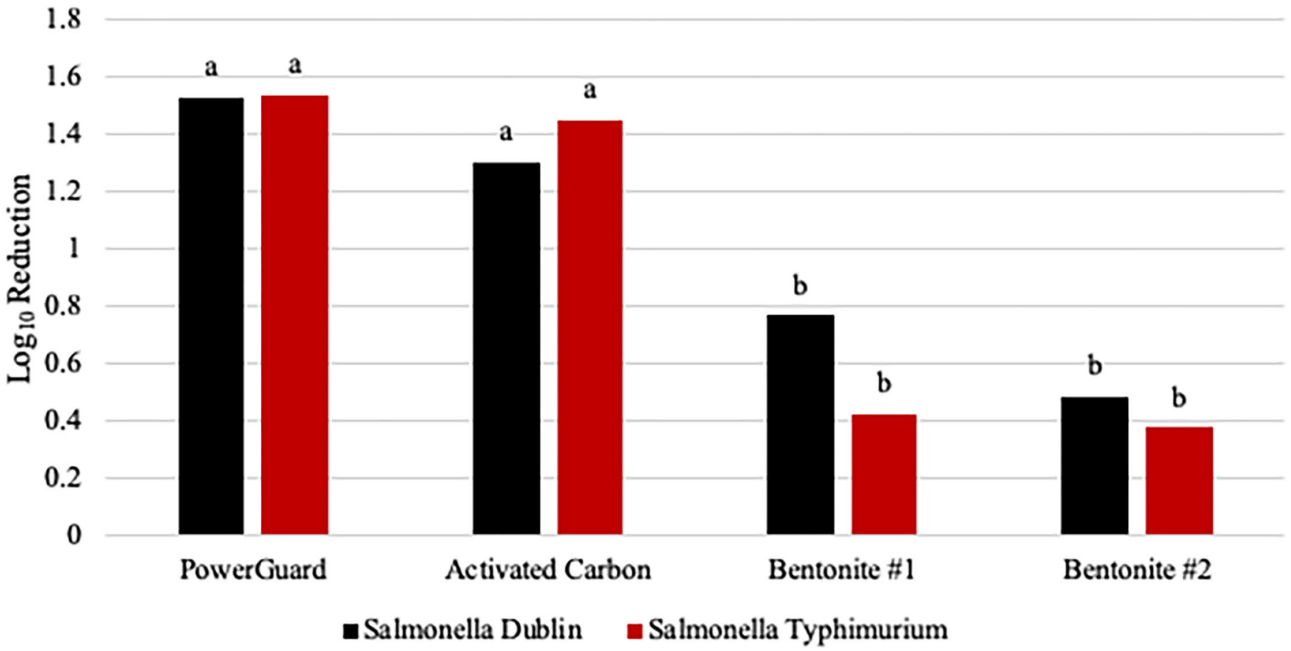

FIGURE 1 | Measures of the capacity for different adsorbents to reduce bacteria concentrations, reported as $\log_{10}$ reduction, in final filtrate for *Salmonella enterica* serotypes Dublin and Typhimurium. There was no adsorbent treatment × *S. enterica* serotype interaction ($P = 0.696$). However, there was an adsorbent treatment difference for $\log_{10}$ reduction of both *Salmonella enterica* serotypes ($P < 0.0001$). The largest scanning electron microscopy (SEM) is reported as $\log_{10}$ reduction and is 0.2123. Bars with different superscripts are statistically different ($P < 0.05$).

compared to the ST and PG0.5 treatments, and PG0.05 and PG0.25 treatments were intermediate. There was a tendency for a treatment × time interaction for hematocrit ($P = 0.0837$; **Figure 5**). There was a treatment difference for hemoglobin ($P = 0.0003$), where the ST and PG0.5 treatments had decreased hemoglobin concentrations when compared to all other treatments. There was a tendency for a treatment × time interaction for reticulocyte counts ($P = 0.1$; **Figure 6**). There was no treatment difference for platelet count ($P = 0.163$). There were treatment differences in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) ($P \leq 0.019$), where the ST and PG0.5 had decreased MCV and MCH when compared to all other treatments. There was a treatment × time interaction for total leukocyte count ($P = 0.0074$; **Figure 7**), where leukocyte counts were increased post-infection for the ST pigs compared to all other treatments. There was a treatment × time interaction for neutrophil count ($P = 0.0026$; **Figure 8**), where neutrophil counts were increased post-infection for the ST pigs compared to all other treatments. There was a treatment × time interaction for monocyte count ($P = 0.0048$; **Figure 9**), where monocyte counts were increased post-infection for the ST pigs compared to all other treatments. There was no treatment difference for lymphocyte count ($P = 0.512$).

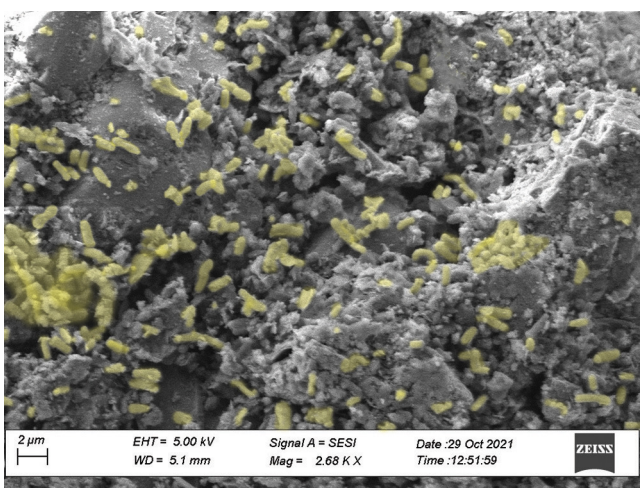

FIGURE 2 | The reported electron micrograph shows *Salmonella* Typhimurium, highlighted in yellow, adsorbed to the surface of PowerGuard. SEM images were taken by Zeiss crossbeam 540 at 5 kiloelectron volts (kV) using secondary electrospray ionization detector (Zeiss Meditec Inc., Dublin, CA, USA). Samples were observed at the Texas Tech University Microscopy Core Laboratory by SEM with the help of Dr. Zhao.

TABLE 2 | Performance effects of a ceramic particle fed to pigs after a *Salmonella enterica* serotype typhimurium infection.

	Treatments[1,2]					Largest	Fixed Effects		
Item	CON	ST	PG0.05	PG0.25	PG0.5	SEM	Trt	Time	Trt*Time
							P ≤		
Initial/weaned body weight, kg	6.69	8.03	7.06	7.39	7.80	0.466	0.201	…	…
Final body weight, kg	12.46	12.86	12.74	13.84	11.69	1.322	0.767	…	…
Feed disappearance, kg/d[3]	0.581	0.386	0.515	0.554	0.363	0.056	0.077	<0.0001	0.028
Average daily gain, kg/d[3]	0.301	0.205	0.283	0.307	0.169	0.058	0.312	<0.0001	0.387
Feed disappearance to gain, kg[3]	1.508	1.354	1.256	1.317	1.364	0.302	0.472	0.007	0.059

[1] *Treatments include (1) Uninfected Control, no ST administered and no treatment in the diet (CON); (2) Infected Control, infected with ST on day 7 but no treatment in the diet (ST); (3) PG0.05, infected with ST on day 7 and 0.05% PG in the diet (PowerGuard ceramic particle, MB Nutritional Sciences, Lubbock, TX); (4) PG0.25, infected with ST on day 7 and 0.25% PG in the diet; (5) PG0.5, infected with ST on day 7 and 0.5% PG in the diet.*

[2] *Rows with differing superscripts indicate treatment differences with P < 0.05.*

[3] *Repeated measures analysis reports the LSMEANS for each respective treatment group from a separate analysis of each respective variable utilizing the summed days 0–21 data. These are more accurate representative LSMEANS than the LSMEANS from the repeated measures analysis due to, in part, using unequally spaced time points: days 0–7; days 8–10; days 11–14; days 15–21.*

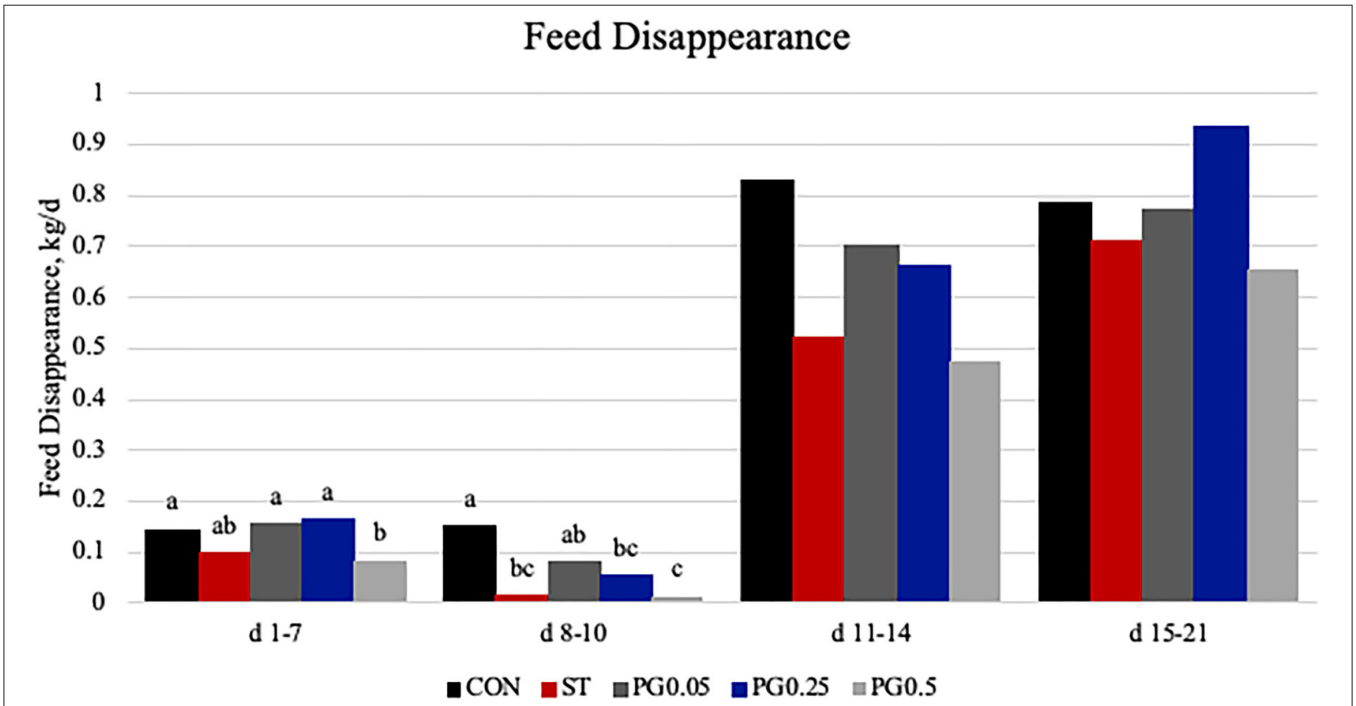

FIGURE 3 | Feed disappearance was measured on days 7, 10, 14, and 21. There was a treatment × time interaction ($P = 0.0283$). There was a tendency for a difference from days 1 to 7 ($P = 0.0826$), where the PG0.5 treatment was decreased when compared to the CON, PG0.05, and PG0.25 treatments ($P \leq 0.065$). There was a treatment difference on days 8–10 ($P = 0.0023$), where CON was greater than all other treatments and PG0.05 was greater than both the ST and PG0.5 treatments ($P \leq 0.0353$). The largest SEM per time point is expressed as kg and includes 0.026, 0.082, 0.1586, and 0.1248 for days 1–7, 8–10, 11–14, and 15–21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

Health

Measures of health are reported in **Table 4**. There was a tendency for treatment difference in the fecal score ($P = 0.087$), where the ST and PG0.5 treatments tended to have increased fecal scores when compared to the CON and PG0.25 treatments. There were treatment differences for both fecal shedding in RV and TT broth ($P < 0.0001$), where the CON treatment shed less ST in feces than every other treatment. There was no difference among treatments for the presence of ST in either lymph node tissue or ileum tissue samples ($P \geq 0.311$). There was a treatment × time interaction for intraperitoneal temperature ($P < 0.0001$; **Figure 10**), where the addition of PG to the diet attenuated the post-infection febrile response.

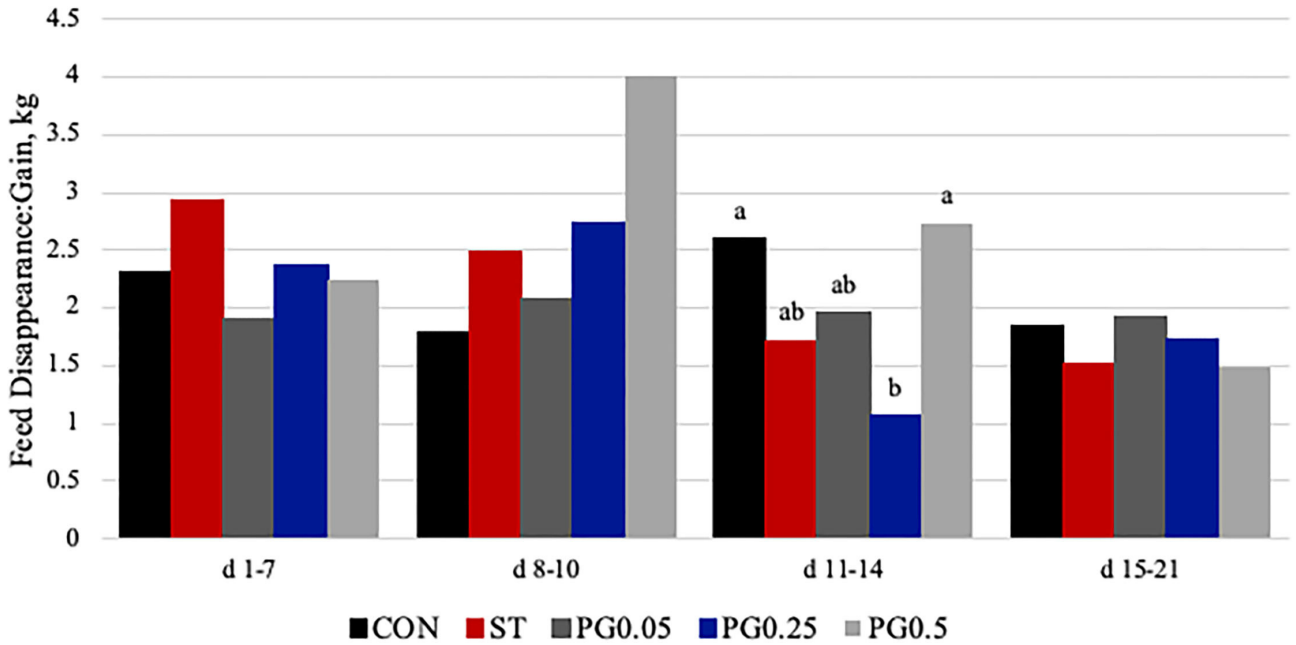

FIGURE 4 | Feed disappearance to gain was calculated from the feed disappearance and change in weight gain from days 1 to 7, 8 to 10, 11 to 14, and 15 to 21. There was a tendency for a treatment × time interaction ($P = 0.0593$). On days 11–14, there was a tendency for treatment difference ($P = 0.0528$), where treatment PG0.25 had decreased FD:G when compared to both PG0.5 and CON treatments ($P \leq 0.0123$). Largest SEM per time point is reported in kg and include 0.8118, 0.9323, 0.6647, 0.3707 for days 1–7, 8–10, 11–14, and 15–21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

TABLE 3 | Hematological effects of a ceramic particle fed to pigs after a *S. enterica* serotype typhimurium infection.

	Treatments[1,2,3]					Largest	Fixed effects		
	CON	ST	PG0.05	PG0.25	PG0.5	SEM	Trt	Time	Trt*Time
Item							*P* ≤		
Hematocrit, %	34.9	29.9	35.5	35.0	29.1	1.30	0.0004	<0.0001	0.084
Hemoglobin, g/dL	9.44^{a}	8.06^{b}	9.69^{a}	9.54^{a}	8.02^{b}	0.337	0.0003	<0.0001	0.369
Red blood cell count (10^9/mL)	7.18^{a}	6.74^{bc}	7.10^{ab}	6.98^{ab}	6.56^{bc}	0.164	0.030	<0.0001	0.274
Mean corpuscular volume (MCV), fl	48.5^{a}	44.4^{b}	50.1^{a}	50.2^{a}	44.2^{b}	1.67	0.015	0.0001	0.522
Mean corpuscular hemoglobin, pg	13.2^{a}	12.0^{b}	13.7^{a}	13.7^{a}	12.2^{b}	0.46	0.019	<0.0001	0.760
Reticulocyte, 10^6/mL	179	113	134	155	104	28.7	0.219	0.0002	0.100
Platelets, 10^6/mL	642	671	634	577	848	89.4	0.163	0.779	0.474
Total leukocyte count (10^6/mL)	17.3	22.7	19.4	19.3	18.2	1.65	0.164	<0.0001	0.007
Neutrophils, 10^6/mL	6.2	9.3	7.9	7.3	7.7	0.93	0.188	<0.0001	0.003
Monocytes, 10^6/mL	0.95	1.74	1.33	1.25	1.20	0.133	0.002	<0.0001	0.005
Lymphocyte, 10^6/mL	10.0	11.6	10.1	10.5	9.2	1.03	0.512	<0.0001	0.618

[1]*Treatments include (1) Uninfected Control, no ST administered and no treatment in the diet (CON); (2) Infected Control, infected with ST on day 7 but no treatment in the diet (ST); (3) PG0.05, infected with ST on day 7 and 0.05% PG in the diet (PowerGuard ceramic particle, MB Nutritional Sciences, Lubbock, TX); (4) PG0.25, infected with ST on day 7 and 0.25% PG in the diet; (5) PG0.5, infected with ST on day 7 and 0.5% PG in the diet.*

[2]*Rows with differing superscripts indicate treatment differences with P < 0.05.*

[3]*Repeated measures analysis reports the LSMEANS for each respective treatment group using data from hematological measures from the following time points: 0, 7, 10, 14, and 21.*

Histology

Ileal histology data are reported in **Table 5**. There was a treatment difference for villi length ($P = 0.0482$) where the PG0.05 treatment had the shortest villi compared to all other treatments. There was no treatment difference in crypt depth ($P = 0.213$). There was a treatment difference in villi length:crypt depth

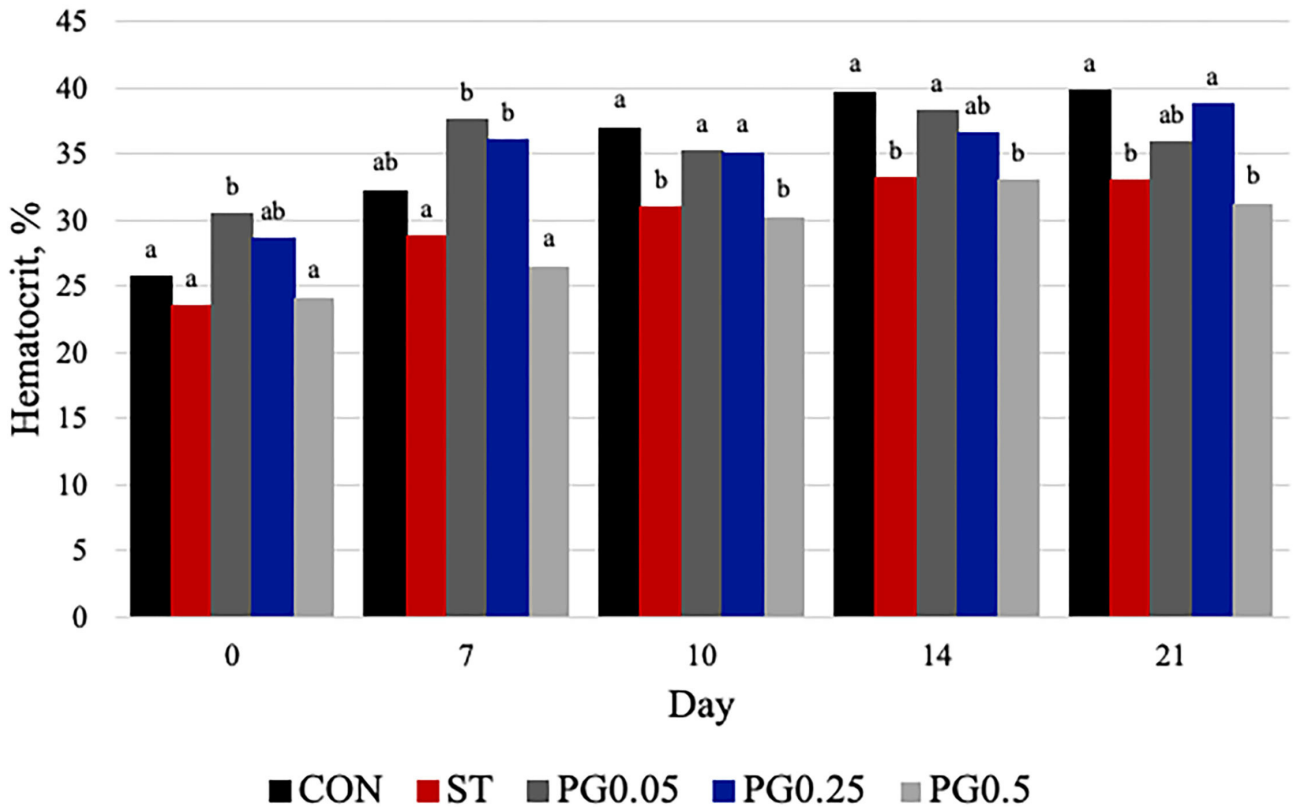

FIGURE 5 | Hematocrit was measured on days 0, 7, 10, 14, and 21. There was a tendency for a treatment × time interaction ($P = 0.0837$). There were treatment differences at day 0 ($P = 0.0083$), day 7 ($P = 0.0057$), day 10 ($P = 0.272$), day 14 ($P = 0.019$), and day 21 ($P = 0.0037$). On day 0, treatment PG0.05 was increased when compared to ST, CON, and PG0.5 ($P \leq 0.023$); PG0.25 was increased when compared to ST and PG0.5 ($P \leq 0.025$). On day 7, treatment PG0.05 was increased compared to CON, ST, and PG0.5 ($P \leq 0.0135$); PG0.25 was increased compared to ST and PG0.5 ($P \leq 0.0135$); CON was increased when compared to ST ($P = 0.063$). On day 10, treatments PG0.5 and ST were decreased compared to all other treatments ($P \leq 0.496$). On day 14, treatment PG0.05 was increased compared to ST and PG0.5 ($P \leq 0.0502$); CON was increased when compared to ST and PG0.5 ($P \leq 0.0095$). On day 21, treatment PG0.5 was decreased when compared to PG0.05, PG0.25, and CON ($P \leq 0.0695$); ST was decreased when compared to CON and PG0.25 ($P \leq 0.0245$). Largest SEM are reported as percentage and include 2.036, 3.192, 2.423, 2.362, and 2.442 for days 0, 7, 10, 14, and 21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

($P = 0.0115$) where the PG0.05 treatment had a decreased ratio compared to all other treatments. There was a treatment difference for villus blunting score ($P = 0.0169$) where the CON and PG0.25 treatments had decreased blunting scores, ST and PG0.5 had intermediate scores, and the PG0.05 treatment had an increased villus blunting score. There was no treatment difference in villus epithelium score ($P = 0.7053$). There was a treatment difference for lacteal dilation score ($P = 0.0383$), where the ST treatment had an increased score when compared to all other treatments. There was no treatment difference for goblet cell count ($P = 0.7856$). There was a treatment difference for intraepithelial lymphocyte count ($P = 0.0017$), where the ST treatment had increased cell counts when compared to all other treatments. There were no treatment differences for lamina propria lymphocyte, neutrophil, or eosinophil cell counts ($P \geq 0.1425$).

Liver histology data are presented in **Table 6**. There was no treatment difference in the hazy vacuolization score ($P = 0.159$). There was a treatment difference in connective tissue prominence score ($P = 0.009$), where the CON treatment had a decreased score and PG0.5 had an intermediate score compared to all other treatments. There was a treatment difference for lymphocyte foci score ($P = 0.02$), where the CON treatment had a decreased score, PG0.25 had an increased score, and all other treatments were intermediate. There was no treatment difference for the lymphocyte foci area ($P = 0.199$). There was a treatment tendency for neutrophil score ($P = 0.052$), where the CON treatment had a decreased score and PG0.25 had an increased score, with all other treatments intermediary.

DISCUSSION

The current study investigated the *in vitro* capacity for adsorption of *Salmonella enterica* serotypes Dublin and Typhimurium, and the *in vivo* dose-response of supplementation of a porous ceramic particle, PG, on the health, recovery, and histopathology

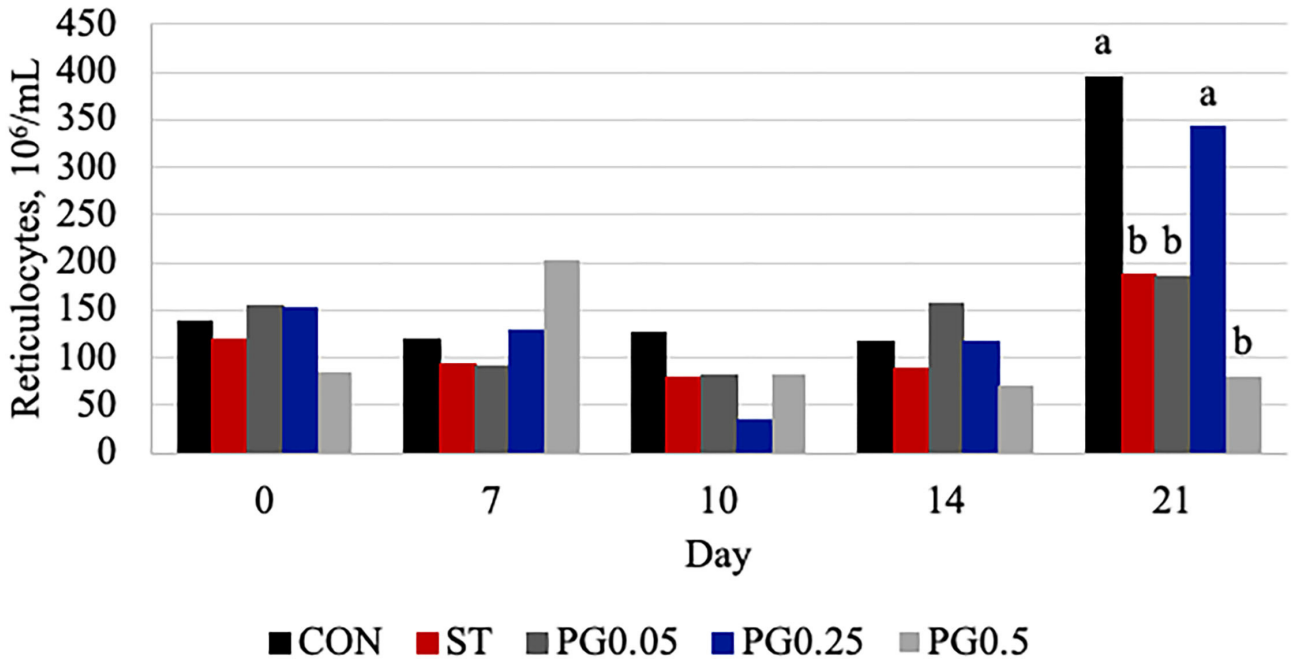

FIGURE 6 | Reticulocyte count was measured on days 0, 7, 10, 14, and 21. There was a tendency for a treatment × time interaction ($P = 0.1004$). There was a treatment difference on day 21 ($P = 0.0013$), where CON and PG0.25 were increased when compared to all other treatments ($P \leq 0.0426$). The largest SEM per time point is reported as 10^6/ml and include 82.72, 90.69, 65.83, 57.46, and 87.72 for days 0, 7, 10, 14, and 21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

of weanling pigs infected with *Salmonella* typhimurium. The microporosity and extensive available surface area of ceramic particles are contributing factors for effective adsorption to bacteria, particularly pathogenic species. Unprocessed montmorillonite was shown to adsorb, per gram, a total of 4×10^9 CFU of *S. enterica*. Similar to the current study, adsorption of *S. enterica* occurred exclusively on the external surface of the clay (Pardo et al., 2020). Clay adsorption of *S. enterica* can depend on the interlayer cations present, which can differ among and within mineral deposits. The lipopolysaccharide molecules present in gram-negative bacteria, like *S. enterica*, have a strong negative charge which can preferentially bind to the Ca^{2+} and Na^{+} cations present in the interlayer space in the structure of montmorillonite clays (Unuabonah et al., 2018). When clays are thermally processed into ceramics the surface micropores increase in size and number, thus the adsorption surface area potential increases (Schell et al., 1993; Diaz et al., 2002).

The adsorption capacities for processed clay, like PG, to remove potential toxins and bacteria from the environment and the gastrointestinal tract are still being determined. A chemically processed montmorillonite clay was utilized as a soil amendment to determine its capacity to adsorb the hydrophobic pesticide, Dieldrin. When treatment was applied, it achieved a 70% reduction in the amount of Dieldrin recovered in soil. The adsorption to Dieldrin was theorized to occur *via* the interlayer surfaces of the clay (Hearon et al., 2020). An *in vitro S.* typhimurium adsorption study was conducted utilizing a Cu-montmorillonite clay. Clay at 0.05 g/mL adsorbed the best to *S.* typhimurium included at 4×10^8 CFU and was the most effective dose of clay for sterilization (Ting et al., 2020). The capacity for clay or ceramic particles to reduce bacteria load is critically important when considering the farm-to-consumption food safety pathway. Results from a quantitative microbiological risk assessment (QMRA) in the EU indicate a reduction in one to two log base 10 of *Salmonella* on the pre-chill carcass can potentially prevent up to 90% of human *Salmonella* infections of pork product origin (EFSA, 2011). In the current study, the *in vitro Salmonella* adsorption analyses determined that PG and activated carbon could reduce *Salmonella* typhimurium concentration by approximately 1.5 $\log_{10}$ when initial bacteria concentrations began at 2×10^6 CFU/mL. This reduction is biologically relevant when considering the impacts of bacteria reduction on pre-harvest carcasses. Similarly, PG and activated carbon were also capable of reducing the final concentration of *Salmonella* dublin by 1.5 and 1.3 $\log_{10}$, respectively, when initial *Salmonella* dublin concentrations began at 2×10^6 CFU/mL. Both adsorption assays included two unprocessed commercial bentonite clays as comparative controls, and the adsorption

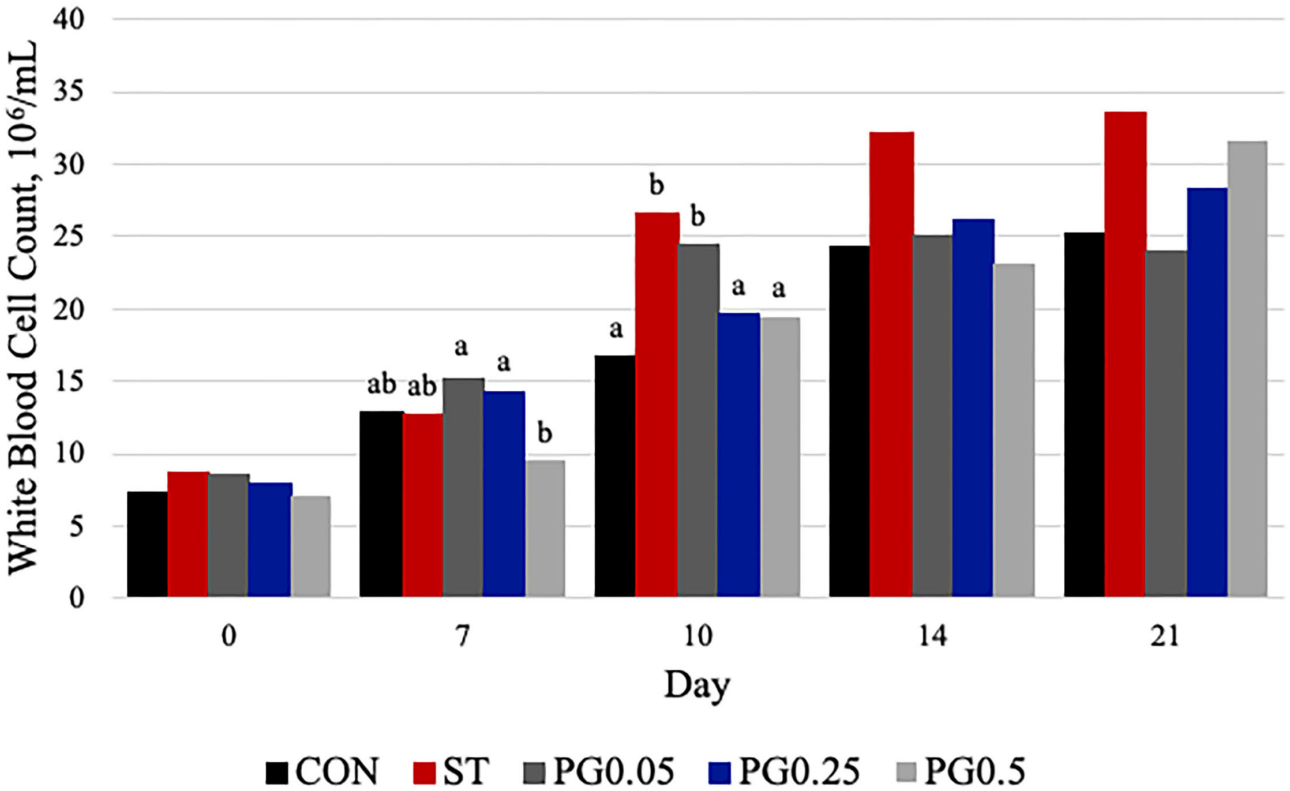

FIGURE 7 | Leukocyte count was measured on days 0, 7, 10, 14, and 21. There was a treatment × time interaction ($P = 0.0074$). There were treatment differences on day 7 ($P = 0.0468$) and day 10 ($P = 0.0148$). On day 7, treatment PG0.5 was decreased when compared to the CON, PG0.05, and PG0.25 treatments ($P \leq 0.0116$). On day 10, the ST and PG0.05 treatments were increased when compared to all other treatments ($P \leq 0.0247$). The largest SEM per time point is expressed as 10^6/mL and include 1.506, 1.954, 2.959, 3.601, and 4.648 for days 0, 7, 10, 14, and 21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

capacity was less than one $\log_{10}$ for both bacteria strains. Data from the literature suggests the mechanisms for adsorption are similar between processed clays and activated carbon products and were shown to have similar capacities in the current data (van der Mei et al., 2008; Chu et al., 2013; Annan et al., 2018; Schmidt et al., 2019; Ting et al., 2020). This increase in adsorption capacity for biotoxins and bacteria make ceramic particles a viable option for feed additives to lower the exposure load of infectious or zoonotic pathogens, such as *Salmonella sp.,* that food animals encounter.

The *S.* typhimurium infection in the current study decreased feed disappearance and numerically decreased ADG immediately following the infection. Supplementing PG at 0.05% of the diet prevented the decrease in feed disappearance when compared to the uninfected controls from days 8 to 10. The PG0.25 had improved feed disappearance:gain when compared to the CON and PG0.5 pigs from days 11 to 14. The PG0.5 treatment did not improve any aspect of performance. It was reported that the inclusion of clay at increased feed rates from 0.5 to 3% of the diet can decrease performance and may adsorb micronutrients or interfere with nutrient absorption (Elliot et al., 2020). Similar data supplementing montmorillonite clay were reported in both healthy and infected pigs (Song et al., 2012; Liu et al., 2020). However, other data reported no difference in production performance when healthy, uninfected pigs were supplemented with a montmorillonite clay (Thacker, 2003; Kim et al., 2006; Almeida et al., 2013; Holanda and Kim, 2020). This suggests clay supplementation may be the most beneficial when animals are under specific conditions, including stressors like weaning, transport, or comingling, and times of natural increased exposure to more pathogens and their biotoxins.

Diarrhea is common in pigs infected with *Salmonella enterica*, and the ST infection in the current study caused diarrhea. Supplementing PG at 0.25% of the diet prevented diarrhea, whereas the lower or greater doses of PG at 0.05 and 0.5% of the diet, respectively, did not prevent the pigs from developing diarrhea. The diarrheal response of a host to infection with *S.* typhimurium is due to the influx of neutrophils into the

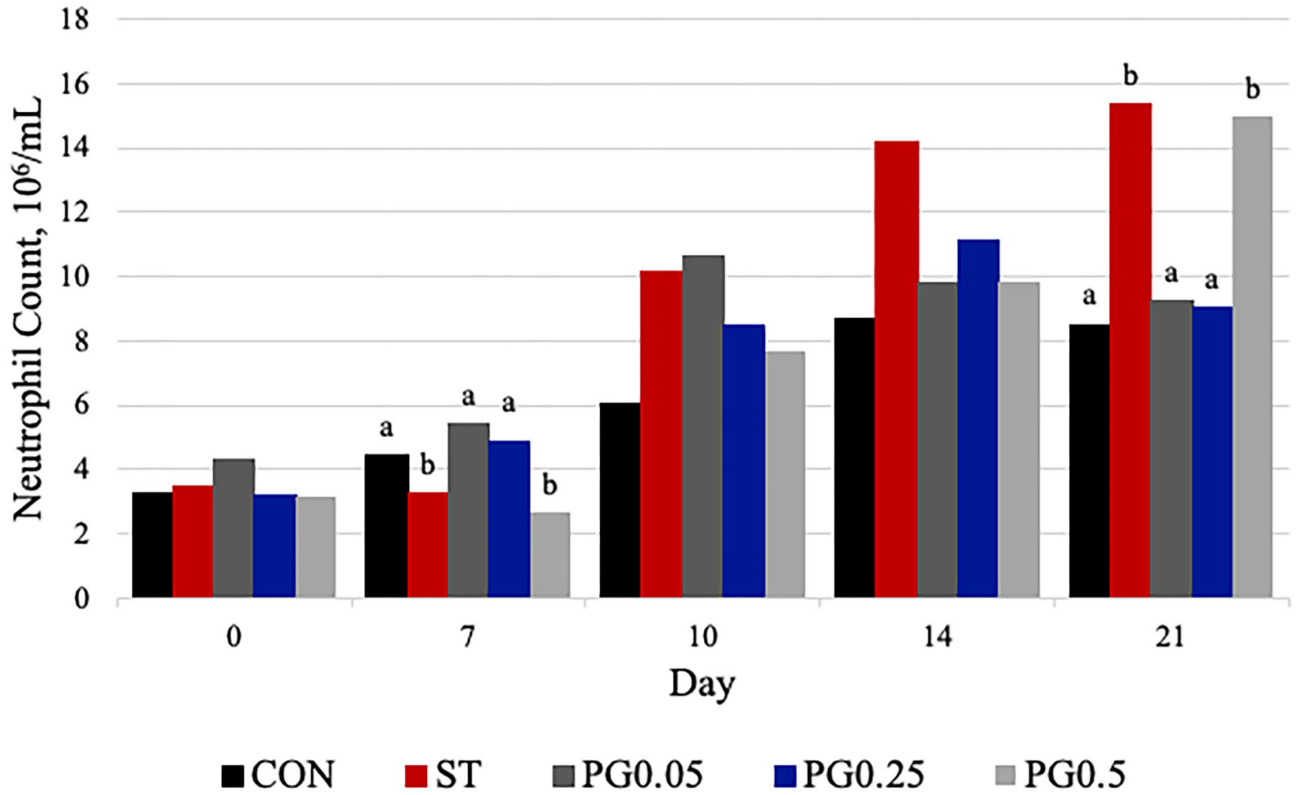

FIGURE 8 | Neutrophil count was measured on days 0, 7, 10, 14, and 21. There was a treatment × time interaction ($P = 0.0026$). There were treatment differences on day 7 ($P = 0.0023$) and day 21 ($P = 0.0116$). On day 7, treatments PG0.05 and PG0.25 were decreased when compared to ST and PG0.5 ($P \leq 0.0078$); CON was also increased when compared to PG0.5 ($P = 0.0177$). On day 21, treatments PG0.05, PG0.25, and CON were decreased when compared to the ST and PG0.5 treatments ($P \leq 0.0313$). The largest SEM is reported per time point as 10^6/ml and include 1.066, 0.7756, 2.243, 2.278, and 2.692 for days 0, 7, 10, 14 and 21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

gastrointestinal tract resulting in lumen edema (Lou et al., 2019). These data suggest that supplementing the diet with PG at 0.25% of the diet reduced the local inflammatory response in the intestinal tract. The exact reason why the PG0.05 or PG0.5 pigs did not have improved fecal consistency compared to the ST-infected pigs is not known especially since both of those treatments did reduce the early neutrophilia that was observed in the ST pigs after infection.

Hematological changes expected during an *S.* typhimurium infection include an increase in total leukocytes, which is primarily driven by neutrophilia. Additionally, the infection commonly causes a decrease in hemoglobin and red blood cell counts and morphology. In the current study, the *S.* typhimurium infection caused an increase in total leukocytes and neutrophil counts over the duration of the study in ST infected pigs as expected; however, the increase in total leukocytes and neutrophil counts was more moderate and transient when PG was supplemented at 0.05, 0.25, or 0.5% of the diet. In agreement, supplementing clay to pigs without any signs of infection or disease also resulted in a decrease in total leukocyte counts at both 25 and 70 days of age (Bederska-Lojewska and Pieszka, 2019). There was a delayed increase in neutrophil counts in the PG0.5 treatment on day 21. Furthermore, the RBC profile, hemoglobin concentrations, red blood cell counts, and red blood cell morphology in the PG0.05 and PG0.25 treatments were similar to the CON pigs, whereas the ST group was similar to the PG0.5 pigs and indicative of infection and inflammation (Barba-Vidal et al., 2017). These data further support that PG at 0.05 and 0.25 attenuated the infection and inflammatory response associated with the ST infection. The exact mechanisms for the delayed increase in neutrophil counts and that the red blood cell data were not different than the ST in the PG0.5 are not known.

The intraperitoneal temperature began to increase in ST-infected pigs around 12 h post-infection, which was previously documented (Balaji et al., 2000; Jenkins et al., 2004; Price et al., 2010). The addition of PG at any dose resulted in an intermediary reduction in febrile response for the first

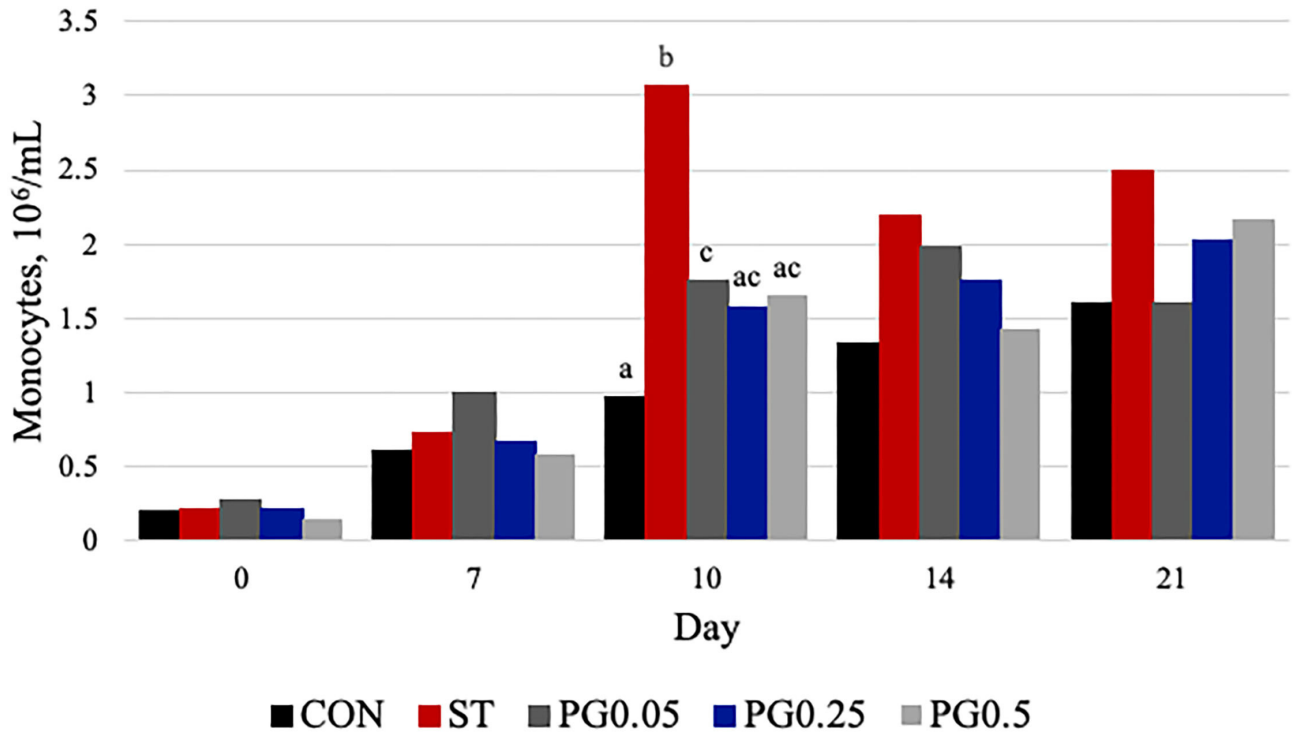

FIGURE 9 | Monocyte count was measured on days 0, 7, 10, 14, and 21. There was a treatment × time interaction ($P = 0.0048$). There was a treatment difference on day 10 ($P = 0.0003$), where the ST treatment was increased when compared to all other treatments ($P \leq 0.0038$). The largest SEM is reported as 10^6/ml and include 0.059, 0.2, 0.3925, 0.3714, and 0.5068 for days 0, 7, 10, 14, and 21, respectively. Bars with different superscripts are statistically different ($P < 0.05$).

TABLE 4 | Health effects of a ceramic particle fed to pigs after a *S. enterica* serotype typhimurium infection.

	Treatments[1,2]					Largest	Fixed effects		
	CON	ST	PG0.05	PG0.25	PG0.5	SEM	Trt	Time	Trt*Time
Item							*P* ≤		
Fecal score, average by week	0.628[a]	1.046[b]	0.912[ab]	0.586[a]	0.975[ab]	0.155	0.087	<0.0001	0.1665
Fecal shedding RV, % days positive[3]	0.0[a]	41.1[b]	32.4[b]	32.5[b]	37.5[b]	0.048	<0.0001	…	…
Fecal shedding TT, % days positive[3]	7.5[a]	73.3[b]	63.8[b]	64.2[b]	64.2[b]	0.063	<0.0001	…	…
Ileum RV, % pigs positive[4]	22.9	17.1	17.1	20.0	22.9	…	0.712	…	…
Ileum TT, % pigs positive[4]	23.5	17.7	20.6	17.7	20.6	…	0.483	…	…
Lymph node RV, % pigs positive[4]	25.0	12.5	18.8	21.9	21.9	…	0.563	…	…
Lymph node TT, % pigs positive[4]	28.6	14.3	21.4	17.9	17.9	…	0.311	…	…
Intraperitoneal temperature, °C	39.24	39.37	39.26	39.49	39.21	0.129	0.413	0.995	<0.0001

[1] *Treatments include (1) Uninfected Control, no ST administered and no treatment in the diet (CON); (2) Infected Control, infected with ST on day 7 but no treatment in the diet (ST); (3) PG0.05, infected with ST on day 7 and 0.05% PG in the diet (PowerGuard ceramic particle, MB Nutritional Sciences, Lubbock, TX); (4) PG0.25, infected with ST on day 7 and 0.25% PG in the diet; (5) PG0.5, infected with ST on day 7 and 0.5% PG in the diet.*

[2] *Rows with differing superscripts indicate treatment differences with $P < 0.05$.*

[3] *Two fecal swabs were collected from fresh feces from each crate beginning on day 7 prior to ST administration. One swab was placed directly into Rappaport-Vassiliadis (RV) broth (Thermo Fischer Scientific, Waltham, MA, USA) and one swab was placed directly into Difco Tetrathionate (TT) broth (Thermo Fischer Scientific, Waltham, MA, USA). Fresh fecal swabs were collected every morning from day 7 through day 21 by two trained individuals only swabbing fresh feces. If no fresh feces were present, a fecal swab was taken directly from the rectum for that sample day. The fecal swabs in the RV broth were incubated overnight at 42°C and the TT broth swabs were incubated overnight at 37°C, then all samples were streak plated on Xylose Lysine Deoxycholate Agar (XLD) (Thermo Fischer Scientific, Waltham, MA, USA). The plates were then incubated overnight at 37°C to determine if there was Salmonella colony growth, by examining the presence or absence of black colonies. Samples were recorded as either positive or negative and colony counts were not conducted.*

[4] *All direct plate counts on XLD agar were negative, and all samples were stomached in sterile 1X PBS at a 1:10 dilution.*

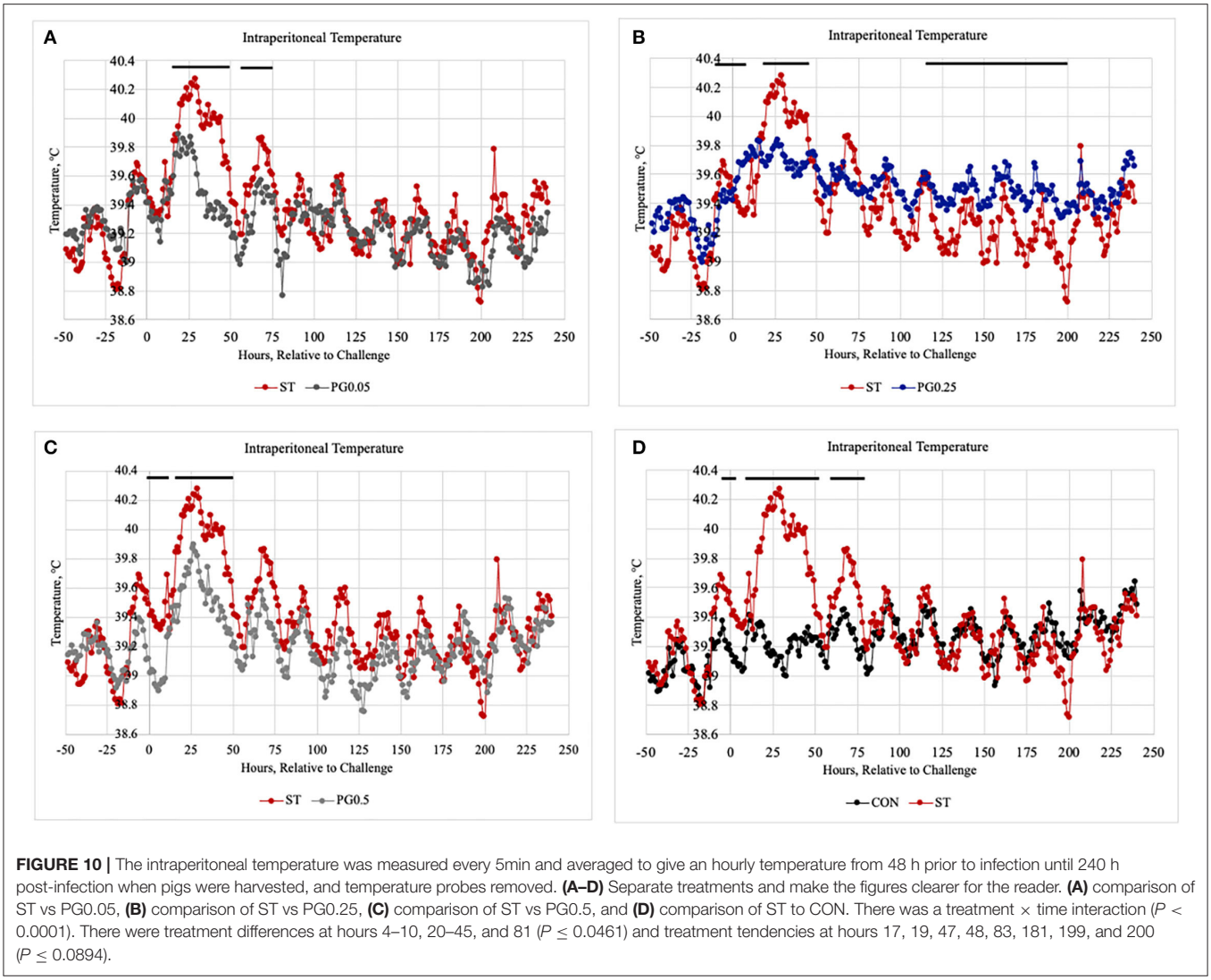

FIGURE 10 | The intraperitoneal temperature was measured every 5min and averaged to give an hourly temperature from 48 h prior to infection until 240 h post-infection when pigs were harvested, and temperature probes removed. **(A–D)** Separate treatments and make the figures clearer for the reader. **(A)** comparison of ST vs PG0.05, **(B)** comparison of ST vs PG0.25, **(C)** comparison of ST vs PG0.5, and **(D)** comparison of ST to CON. There was a treatment × time interaction ($P < 0.0001$). There were treatment differences at hours 4–10, 20–45, and 81 ($P \leq 0.0461$) and treatment tendencies at hours 17, 19, 47, 48, 83, 181, 199, and 200 ($P \leq 0.0894$).

50 h post-infection, whereas all PG treatment groups had reduced febrile responses when compared to the ST pigs but increased temperatures when compared to the CON pigs. Similar responses in temperature were observed in prior *S.* typhimurium infection models when other nutraceuticals were supplemented (Spiehs et al., 2008; Price et al., 2010; Liang et al., 2020). Taken together, the improved performance, reduced diarrhea, attenuated changes in hematology, and reduced febrile responses in pigs supplemented with PG at 0.25% of the diet indicate that PG adsorbed some of the ST and reduced the local and systemic inflammatory responses to the ST infection. Furthermore, supplementing PG at a lower inclusion, 0.05% of the diet, also likely adsorbed some of the ST and reduced many of the systemic inflammatory responses.

Shedding data in the current study was collected by swabbing fresh feces each day and enriching the swabs overnight, revealing only positive or negative results, and there were no differences among the ST-infected treatment groups. No quantification was done on fecal ST swabs in the current study, but data from another pig *S.* typhimurium infection revealed only 8% of pigs had quantifiable levels of *S.* typhimurium in feces for 8 days post-infection (Barba-Vidal et al., 2017). It is important to note that fecal shedding results from the enrichment media show that the CON group did not shed any ST from the RV enrichment and shed statistically less ST in the TT enrichment. These results suggest that the biosecurity measures used in the current study were reasonably successful in preventing horizontal transmission of the ST infection to the negative control group. The presence of ST in the ileum and lymph nodes of CON pig at harvest may be explained by exposure to environmental *Salmonella spp.* prior to initiation of the study. In fact, QMRA on *Salmonella spp.* indicate that interventions in sow farms and farrowing crates may have a greater impact on reducing the risk for *Salmonella spp.* infections than during the grow to finish phase (EFSA, 2011). The propensity for *S.* typhimurium to survive within tissues, such as these is what makes it such an important food safety risk. The prevalence of *S.* typhimurium in the lymph nodes and the active shedding during transport are both risk factors for the harvest facility to contaminate food. A QMRA on *Salmonella* in the harvest plant and in breeder pigs was

TABLE 5 | Ileum histopathological effects of a ceramic particle fed to pigs after a *S. enterica* serotype typhimurium infection.

	Treatments[1,2]					Largest	Fixed Effects
	CON	ST	PG0.05	PG0.25	PG0.5	SEM	Trt
Item							*P* ≤
Villi length, μm	417[a]	414[a]	346[b]	432[a]	430[a]	54.2	0.048
Crypt depth, μm	312	278	306	301	306	27.4	0.213
Villi length:crypt depth	1.34[a]	1.51[a]	1.13[b]	1.44[a]	1.40[a]	0.33	0.012
Villus blunting score[3]	0.43[ac]	1.00[ab]	1.34[b]	0.25[c]	1.00[ab]	0.64	0.017
Villus epithelium score[3]	0	0.17	0.34	0.13	0.25	0.46	0.705
Lacteal dilation score[3]	0.13[a]	0.67[b]	0.16[a]	0.37[ab]	0.0[a]	0.39	0.038
Goblet cell count[4]	0.13	0.17	0.15	0.15	0.15	0.07	0.786
Intraepithelial lymphocyte count[4]	8.75[a]	14.17[b]	7.83[a]	7.50[a]	6.63[a]	3.21	0.002
Lamina propria lymphocyte count[4]	1.75	2.34	1.83	2.37	3.13	1.11	0.143
Lamina propria neutrophil count[4]	0.63	0.66	9.33	0.5	0.63	0.57	0.841
Lamina propria eosinophil count[4]	2.87	4.16	2.83	3.23	3.13	1.49	0.521

[1]*Treatments include (1) Uninfected Control, no ST administered and no treatment in the diet (CON); (2) Infected Control, infected with ST on day 7 but no treatment in the diet (ST); (3) PG0.05, infected with ST on day 7 and 0.05% PG in the diet (PowerGuard ceramic particle, MB Nutritional Sciences, Lubbock, TX); (4) PG0.25, infected with ST on day 7 and 0.25% PG in the diet; (5) PG0.5, infected with ST on day 7 and 0.5% PG in the diet.*

[2]*Rows with differing superscripts indicate treatment differences with P < 0.05.*

[3]*Scoring was on a 0 to 3, scale with 0 = normal tissue; 1 = mild inflammation; 2 = moderate inflammation; and 3 = marked inflammation. Villi blunting: 0 = long, slender villi; 1 = 75% of normal height; 2 = 50% of normal height; 3 = 25% of normal height. Villi epithelium score: 0 = single layer of columnar epithelium; 1 = degeneration, vacuolation, or separation of focal areas of superficial epithelium; 2 = more marked changes and some focal loss of epithelium; 3 = widespread ulceration of the epithelium surface. Lacteal dilation score: 0 = central lacteal represents approximately 25% of the width of the villi; 1 = represents approximately 50% of the villi width; 2 = 75% of the villi width; 3 = dilated to 100% of the villi width. Score data were analyzed as the average of the 5 villi scored and using a normal distribution.*

[4]*Goblet cell count in the crypt and intraepithelial lymphocyte counts are the numbers of cells counted per 50 epithelial cells; Lamina propria counts were the number of each leukocyte (lymphocytes, neutrophils, and eosinophils) observed between crypts in an x40 field. Count data were analyzed using a Poisson distribution, but data are reported as the LS Mean from the model analyzed as a normal distribution.*

TABLE 6 | Liver histopathological effects of a ceramic particle fed to pigs after a *S. enterica* serotype typhimurium infection.

	Treatments[1,2]					Largest	Fixed effects
	CON	ST	PG0.05	PG0.25	PG0.5	SEM	Trt
Item							*P* ≤
Hazy vacuolization score[3]	0.87	2.34	1.17	1.25	1.63	0.45	0.159
Connective tissue prominence score[4]	0.38[a]	2.17[b]	1.0[b]	1.63[b]	1.25[ab]	0.37	0.009
Lymphocyte foci score[5]	0.63[a]	2.17[b]	1.84[b]	2.0[b]	1.5[ab]	0.48	0.020
Lymphocyte foci area, μm[5]	648	1353	1396	1739	759	488	0.199
Neutrophil score[6]	0.63[a]	1.5[b]	0.84[a]	1.88[b]	1.38[ab]	0.48	0.052

[1]*Treatments include (1) Uninfected Control, no ST administered and no treatment in the diet (CON); (2) Infected Control, infected with ST on day 7 but no treatment in the diet (ST); (3) PG0.05, infected with ST on day 7 and 0.05% PG in the diet (PowerGuard ceramic particle, MB Nutritional Sciences, Lubbock, TX, USA); (4) PG0.25, infected with ST on day 7 and 0.25% PG in the diet; (5) PG0.5, infected with ST on day 7 and 0.5% PG in the diet.*

[2]*Rows with differing superscripts indicate treatment differences with P < 0.05.*

[3]*Hazy vacuolization (HV) score ranges from 0-3, with 0 = no HV in zones 1, 2, or 3; 1 = HV in zone 1; 2 = HV present in zones 1 and 2 and is diffuse; 3 = HV present in zones 1, 2, and 3, diffuse and severe.*

[4]*Connective tissue (CT) prominence score ranged from 0 to 3, with a score 0 = no prominent CT; 1 = slightly prominent CT, not diffuse; 2 = more prominent CT, throughout the sample; 3 = all CT present is severe and prominent.*

[5]*Lymphocyte foci score ranged from 0 to 3, with 0 = no lymphocyte aggregates present; 1 = few lymphocyte aggregates present; 2 = multiple lymphocyte aggregates present; 3 = severe and diffuse lymphocyte aggregates throughout the sample. Lymphocyte foci area was measured in μm and conducted on up to 5 foci/samples and averaged per sample.*

[6]*Neutrophil scores ranged from 0 to 3, with 0 = no neutrophils present in lymphocyte aggregates; 1 = <5 neutrophils within lymphocyte aggregates; 2 = between 5 and 10 neutrophils within the lymphocyte aggregates; and 3 = more than 10 neutrophils within the lymphocyte aggregates.*

conducted in the EU, where they determined a strong linear relationship between the lymph node prevalence of *Salmonella* at slaughter and human infection from pork products (EFSA, 2011). The ability of PG at lower inclusion levels, 0.05–0.25% of the diet, to adsorb *Salmonella spp.* in the gastrointestinal tract without any detrimental effects on performance may reduce the total pathogen load that can infect the host, thereby lessening the exposure load that pigs face during the entire pre-harvest stage. This reduction in exposure may have even greater impacts post-harvest by ultimately lowering the contamination of pork

products pre-consumption. Future research should investigate the use of PowerGuard throughout the entire production system to reduce pre-harvest colonization of lymph nodes and fecal shedding of *Salmonella spp*. at harvest.

CONCLUSION

Supplementing PowerGuard at 0.25% of the diet during a *Salmonella* typhimurium infection prevented some of the negative effects on production performance, reduced diarrhea, and improved pathophysiological measures in post-weaned pigs. Further, supplementing PowerGuard at a lower inclusion rate of 0.05% of the diet was intermediately effective at reducing inflammation post *Salmonella* typhimurium infection. Interestingly, supplementing PowerGuard at the greater inclusion rate of 0.5% of the diet did not consistently improve the response of pigs to the *Salmonella* typhimurium challenge. More research needs to be completed to understand this response, although this inclusion rate is greater than what this study seemed to determine as more of the optimum dose of 0.25% of the diet. If pathogen load pre-harvest can be reduced between just 0.5 and 1 $\log_{10}$, the post-harvest contamination potential and the human disease outcomes can be greatly reduced. PowerGuard, a porous commercial ceramic particle, adsorbed *S. enterica* serotypes *in vitro* and reduced disease *in vivo*. Therefore, supplementing PowerGuard may be one intervention strategy along the farm-to-consumption pathway to help mitigate pathogen load and improve food safety.

AUTHOR'S NOTE

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the USDA-ARS, Livestock Issues Research Unit's Institutional Animal Care and Use Committee (IACUC protocol #LIRU-2120S).

AUTHOR CONTRIBUTIONS

ED, KW, NB, RB, JC, and MB contributed to conception and design of the study and performed the animal work. MB performed the statistical analysis, involved in the design of the experiment, consulted on data analyses, and edited the final version of the manuscript. MC assisted on sample analysis and aided in manuscript writing. AP assisted in animal work and formulation of diets. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of Interest: MB has equity ownership in MB Nutritional Sciences LLC.

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