Comparative efficacy of spray-dried plasma and bacitracin methylene disalicylate in reducing cecal colonization by *Salmonella Enteritidis* in broiler chickens

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ABSTRACT Spray-dried plasma (**SDP**) contains immunoglobulins and glycoproteins that possess antibacterial properties. Two floor-pen trials were conducted to determine the efficacy of dietary SDP and bacitracin methylene disalicylate (**BMD**) antibiotic in reducing intestinal colonization by *Salmonella* Enteritidis (**SE**) in broiler chickens. Experiment 1 was a 2-wk, 3 × 2 factorial design consisting of 6 treatments. Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment **BMD** consisted of chicks given unmedicated corn-SBM basal into which BMD was added at 0.055g/kg diet. Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30g/kg diet. Treatments CON-SE, BMD-SE, and SDP-SE consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.46×10^8 CFU SE /mL at 1 day of age. Experiment 2 was a 42-day trial that was similar to Experiment 1 in design, except that chicks were placed on fresh clean litter. On d 3, 7, 14, and 28 post-challenge (**PC**), ceca SE concentration was enumerated on xylose lysine tergitol-4 (**XLT4**) agar. Body weight gain (**BWG**) and feed conversion ratio (**FCR**) were also recorded. Results for d 3 showed that BMD- and SDP-fed chicks had similar ($P > 0.05$) cecal SE (3.39 log $_{10}$ CFU / g and 3.58 log $_{10}$ CFU / g, respectively), but these levels were lower ($P < 0.05$) than that of CON-fed chicks (5.68 log $_{10}$ CFU / g). A similar trend was observed on d 7 and 14 PC. The BMD- and SDP-fed chicks also had higher BWG and FCR ($P < 0.05$) when compared with CON-fed chicks up to d 14. Thereafter, only BMD treatment sustained this growth-promoting effect till d 42 in SE-challenged birds. In conclusion, BMD and SDP showed similar efficacy in reducing cecal *Salmonella* and in mitigating consequent growth-depressing effect(s) in broiler chicks up to 2 wk of age.

Key words: spray-dried plasma, bacitracin methylene disalicylate, *Salmonella* spp., broiler chickens

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INTRODUCTION

Transmission of non-typhoidal *Salmonella* through direct or indirect contact with live poultry has been established as one of the most common causes of human Salmonellosis (a foodborne illness) in the United States (Hale et al., 2012; Scallan et al., 2015; Basler et al., 2016; Nichols et al., 2018; Carrasco et al., 2019). Live poultry can become infected with *Salmonella* spp through vertical transmission from infected hens, or by contamination from the hatchery environment, brooder house environment (litter and ambient air), comingling with infected birds, or consumption of contaminated feed (Roy et al., 2001; Anderson et al., 2016; Buhr et al., 2017; Sharma et al., 2018).

Young chickens (<2 wk) are particularly susceptible to infection by *Salmonella* species. Infection of young chicks may result in malabsorption, impaired growth rate, inefficient feed utilization, and mortality, all which can culminate in economic losses (Neill et al., 1984; Shao et al., 2016; Jazi et al., 2019). However, intestinal *Salmonella* spp. colonization in older birds is frequently asymptomatic, but its persistence throughout the broiler growth cycle is of significant concern to human health. For instance, intestinal and fecal *Salmonella* may contaminate the carcass if intestines rupture during the evisceration stage of processing (Smith et al., 2007; Marin and Lainez, 2009; Buhr et al., 2017). A recent

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surveillance report published by Centers for Disease Control and Prevention showed that *Salmonella* spp. was responsible for 30% of the reported foodborne outbreaks (896 outbreaks) and 35% of total illnesses (23,662). Contaminated chicken and chicken products were implicated as source of infection in majority of these *Salmonella*-associated outbreaks that lead to a total of 4,336 illnesses, 413 hospitalizations, and 1 death (Dewey-Mattia et al. (2018).

Until recently, the control of *Salmonella* spp. in poultry has been accomplished through the administration of in-feed antibiotics that also serve as growth promoters (Broom, 2017). However, the evolvement of antibiotic-resistant bacterial strains, including multidrug resistant *Salmonella* spp. and the risk of their transmission to humans poses significant challenge to the food-safety and public health (Marshall and Levy, 2011; Lin et al., 2013; Cosby et al., 2015; Karp et al., 2017). This have enacted governmental legislation(s) to phase out (or halt) the inclusion of antibiotics (and other antimicrobial drugs) in poultry feed (Food and Drug Administration Veterinary Feed Directive, 2015). Thus, there is a need to develop non-antibiotic alternatives to control intestinal *Salmonella* spp. in poultry. A variety of alternative feed additives such as bioactive biogenics (probiotics, blood-based products, and yeast products) and phytobiotics (prebiotic carbohydrates, organic acids, essential oils, and plant extracts) have been evaluated for their efficacy to improve bird growth performance, immunocompetence, and resistance to disease (Van Immerseel et al., 2006; Meimandipour et al., 2010; Venkitanarayanan et al., 2013; Ortega-Ramirez et al., 2014; Diaz-Sanchez et al., 2015). However, these have yielded variable efficacy and their underlying mechanism (s) of action are still a subject of continuous investigation (Roto et al., 2015; Brown et al., 2017; Salim et al., 2018).

Spray-dried plasma (**SDP**) is a highly nutritive and palatable feed additive which contain functional proteins and essential nutrients that include biologically active peptides (defensins, transferrins), immunoglobulin, albumin, fibrinogen, lipids, growth factors, enzymes, and other components that exert specific biological activities in the intestine (Borg et al., 2002; Peace et al., 2011; Beski et al., 2015; Dietary incorporation of SDP has shown beneficial effects on the gastrointestinal health and growth performance of poultry (Beski et al., 2015; Young and Fasina, 2018; Campbell et al., 2019). For instance, Campbell et al. (2019) reported that incorporation of SDP at up to 40 g/Kg (i.e., 4% level) of the diet during the first 21 d of life, often improved ($P < 0.05$) body weight gain (**BWG**) and feed conversion ratio (**FCR**) of broiler chickens regardless of type of housing (i.e., commercial-type production house or battery cages). Furthermore, a recent study by Jababu et al. (2020) reported similar efficacy for dietary SDP at 30 g/Kg broiler chick diet or bacitracin methylene disalicylate (**BMD**) antibiotic (at 0.055g/kg diet) in improving FCR, maintaining intestinal villi renewal, and increasing jejunal goblet cell density (Jababu et al., 2020). It has been proposed that SPD may exert protective effect on the intestinal epithelium against damage and infections by pathogenic bacteria via increased mucin secretion (McGukin et al., 2011; Moreira Filho et al. 2018; He et al., 2019). However, information is lacking regarding the efficacy of SDP in reducing intestinal *Salmonella* spp. colonization in poultry.

This study compared the potency of porcine SDP supplementation at 30 g/kg diet and BMD antibiotic (at 0.055g/kg diet) to reduce intestinal *Salmonella* spp. colonization in broiler chickens.

Two floor-pen trials were conducted in which broiler chicks given BMD- or SDP-supplemented diets were orally challenged with *Salmonella* Enteritidis – a prevalent poultry associated *Salmonella* serotype within the USA (Shah et al., 2017). Cecal SE loads and growth performance were monitored throughout the duration of each experiment. To the best of our knowledge, this is the first definitive study investigating the efficacy of SDP in reducing intestinal *Salmonella* colonization in broiler chickens.

MATERIALS AND METHODS

All the procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of North Carolina A&T State University.

Description of Salmonella *Strain Used for Experimentation*

Salmonella Enteritidis str. G1 (**SE**) was used to challenge broiler chicks in this study (Shah et al., 2012; Elder et al., 2016; Chiok and Shah, 2019). This *Salmonella* strain is among the most-prevalent poultry-associated *Salmonella* serotypes isolated in the USA (Antunes et al., 2016; Centers for Disease Control and Prevention, 2018; Cox et al., 2019) A spontaneous nalidixic acid resistant mutant derivative of SE was obtained by plating on xylose lysine tergitol-4 (XLT4) media containing 50 μg/mL of nalidixic acid (MP Biomedicals, Irvine, CA) following procedure described previously (Fasina et al., 2008; Fasina et al., 2010). Accordingly, all microbiological media used for the isolation of SE in this study were supplemented with 50 μg nalidixic acid/mL to ensure the growth and recovery of only our resistant marker strain.

Experimental Design, Dietary Treatments, and Animal Husbandry

In Experiment 1, day-old Ross 708 broiler male chicks (n = 380) that have been routinely vaccinated for Marek's disease, New Castle virus, Infectious bursal and Infectious bronchitis virus, were obtained from a commercial hatchery and transported to the Poultry Research Unit at North Carolina A&T State University. To confirm that chicks were free of the nalidixic acid-resistant SE marker strain that was used in this challenge trial, 20 chicks were randomly taken upon arrival, then euthanized by CO_2 asphyxiation, and aseptically necropsied for the removal of ceca into appropriately-

labeled whirlpak filter bags (Nasco, Fort Atkinson, WI). Buffered peptone water (25 mL, **BPW**, Thermo Scientific, Waltham, MA) was pipetted into each whirlpak filter bag, followed by homogenization at medium speed (approx. 230 rpm) for 60 s in a Stomacher 80 Microbiomaster (CamLab, UK). The homogenates were then incubated overnight at 37°C. Next, 1 mL of each homogenate sample was inoculated into sterile tetrathionate (**TT**) and Rappaport-Vassiliadis broths (**RV**; Remel Inc., Lenexa, KS) and incubated for 24 h at 42°C (Thermo Scientific Heratherm Advanced Protocol Microbiological Incubator, Waltham, USA). Following incubation, a loopful (approx. 10 μL) of each RV and TT sample was streaked onto xylose lysine tergitol 4 (**XLT4**; Becton, Dickinson and Company, Sparks, MD) agar plates containing 50 μg/mL of nalidixic acid, and incubated for 48 h at 37°C. Thereafter, presumptive *Salmonella* spp. colonies were isolated and biochemically confirmed by transference into triple sugar iron (**TSI**; Remel Inc., Lenexa, KS) and lysine iron agar (**LIA**; Remel Inc., Lenexa, KS) to determine fermentation end-product formation as described by the USDA Food Safety and Inspection Service Laboratory Guide (USDA, 2019). Samples biochemically confirmed as being *Salmonella* were subjected to serological latex agglutination test using polyvalent O antiserum reactive with serogroups A through I + Vi (Waltman and Gast, 2008).

The remaining 360 chicks were randomly assigned to 6 treatments in a 3 (3 diets) × 2 (*Salmonella* challenge: nonchallenged versus SE-challenged) factorial design as follows: 1) Treatment CON consisted of chicks fed unmedicated corn-soybean meal **(SBM)** basal without SDP; 2) Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which BMD was added at 0.055 g/kg diet; 3) Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which porcine SDP, a kind gift from APC Incorporated (Ankeny, IA), was added at 30 g/kg diet; 4) Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each orally inoculated with 7.46×10^8 colony-forming units (**CFU**) SE /mL at 1 d of age.

Each treatment consisted of 4 replicate floor-pens, with each pen containing 15 chicks. Each pen was equipped with a hanging feeder, a nipple drinker line, and used litter recycled 4 times from a commercial flock. Temperature was set at 92°F from d 1 to d 7, and at 87° F from d 8 to d 14. Photoperiod consisted of continuous (23L:1D) lighting at 30 lux from placement to end of experiment (i.e., 14 d). Experimental diets were formulated to meet the recommendations of the National Research Council (1994). The starter diet was fed as crumbles throughout the experiment. Chicks were given *ad-libitum* access to feed and water throughout the 14-d experiment. Proximate nutrient composition of experimental diets is presented in (Table 1).

Experiment 2 was similar in design to Experiment 1, except that birds were housed on fresh litter and duration of experiment was 6 wk. Briefly, day-old (n = 380) Ross 708 broiler male chicks obtained from the same hatchery as in Experiment 1, were transported to the Poultry Research Unit at North Carolina A&T State University. Upon arrival, 20 chicks were randomly taken and confirmed free of the nalidixic acid-resistant SE marker strain that was used for pathogen challenge, as previously described for Experiment 1. Next, in a 3 (three diets) × 2 (*Salmonella* challenge: nonchallenged vs. SE-challenged) factorial arrangement, the remaining 360 chicks were randomly assigned to 6 treatments that were similar to those described for Experiment 1 (i.e., treatments CON, BMD, SDP, CON-SE, BMD-SE, and SDP-SE).

Each treatment consisted of 4 replicate floor-pens, with each pen containing 15 chicks. Each pen was equipped with a hanging feeder, a nipple drinker line, and fresh clean litter. Temperature was set at 92°F from d 1 to d 7, 87°F from d 8 to d 21, and 77°F from d 22 to d 42. Photoperiod consisted of continuous (23L:1D) lighting at 30 lux from placement to 21 d, and then reduced to 12L:12D lighting from 22 to 42 d. As chicks grew, light intensity was gradually reduced until it reached 5 lux during the last week of experiment. Experimental diets (Table 1) were formulated to meet the recommendations of the National Research Council (1994). The starter diet was fed as crumbles from day 1 to 14 of experiment, and the grower and finisher diets were fed as pellets from d 15 to 28 and d 29 to 42, respectively. Birds were allowed *ad-libitum* access to feed and water throughout the 42-d experiment.

Preparation of Bacterial Inoculum and Salmonella *Challenge*

Frozen stock culture of SE was thawed and 10 μL was inoculated into 10 mL of sterile tryptic soy broth (**TSB**, MP Biomedicals, Irvine, CA). Inoculated broth was incubated overnight at 37°C (Thermo Scientific Heratherm Advanced Protocol Microbiological Incubator, Waltham, MA), and then streaked onto XLT4 agar plates (Becton, Dickinson and Company, Sparks, MD) containing 0.1% nalidixic acid solution (50 μL / mL). Streaked plates were incubated for 48 h at 37°C. Next, a black presumptive colony of SE was inoculated into a tube of 10 mL fresh sterile TSB. The tube was incubated for 24 h, and the resulting culture was used to prepare the challenge inoculum. Accordingly, the SE culture was diluted to contain ~7.5×10^8 CFU / mL using sterile BPW (Thermo Scientific, Waltham, MA). Estimation of total SE cell concentration in the inoculum was done spectrophotometrically at 687 nm with an AccuSkanGo microplate reader (ThermoFisher Scientific, Finland), relative to SE standard curve. Concentration of viable SE cells in the inoculum was then determined by streaking 10 μL onto an XLT4 plate and counting black colonies after incubating the plate overnight at 37°C. Results showed that SE inoculum contained 7.46×10^8 CFU / mL and 7.48×10^8 CFU / mL in Experiments 1 and 2, respectively.

Table 1. Composition of diets for experiments 1 and 2[1] (% "as is").

	Starter diets[1] (D 1 to 14)			Grower diets[1] (D 15 to 28)			Finisher diets[1] (D 29 to 42)		
Ingredient	Control diet	BMD diet	SDP diet	Control diet	BMD diet	SDP diet	Control diet	BMD diet	SDP diet
Corn (7.5% Crude protein)	51.46	51.45	55.94	56.77	56.76	61.24	60.54	60.52	65.01
Soybean meal (47.5% Crude Protein)	40.39	40.40	35.06	35.43	35.43	30.09	31.27	31.27	25.94
Spray-dried plasma (SDP, AP920)	—	—	3.00	—	—	3.00	—	—	3.00
Poultry fat	3.64	3.65	1.96	4.00	4.00	2.31	4.77	4.77	3.08
Limestone	1.07	1.07	1.19	0.64	0.64	0.76	0.53	0.53	0.64
Mono-Dicalcium phosphate	2.03	2.03	1.85	1.84	1.84	1.67	1.66	1.66	1.48
Salt NaCl	0.40	0.40	0.23	0.40	0.40	0.24	0.41	0.41	0.25
Sodium bicarbonate	0.02	0.02	—	0.02	0.02	—	0.02	0.02	—
L-Lysine HCl 98%	0.13	0.13	0.05	0.11	0.11	0.03	0.08	0.08	—
DL-Methionine 99.0%	0.34	0.34	0.28	0.30	0.30	0.24	0.27	0.27	0.21
L-Threonine 98.5%	0.11	0.11	0.04	0.09	0.09	0.02	0.07	0.07	—
NCSU Poultry Vitamin Premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
NCSU Poultry Mineral Premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Bacitracin (Antibiotic, g/kg)	—	0.055	—	—	0.055	—	—	0.055	—
Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Selenium Premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed nutrient composition[4]									
Metabolizable energy (Kcal/kg)	3,117	3,131	3,137	3,283	3,212	3,234	3,287	3,287	3,276
Crude Protein, %	24.63	24.56	24.06	18.81	23.94	22.25	19.31	19.63	21.69
Crude Fat, %	4.74	5.03	4.18	5.06	5.84	5.59	5.12	5.24	5.54
Crude Fiber, %	2.3	2.4	2.3	2.2	2.4	2.2	2.1	2.1	2.4
Ash, %	6.32	6.15	5.35	4.59	5.68	5.19	4.60	4.70	5.48
Calculated nutrient composition									
Total Sulfur Amino Acids, %	1.03	1.03	1.03	0.95	0.95	0.94	0.87	0.87	0.86
Lysine, %	1.42	1.42	1.41	1.27	1.27	1.27	1.13	1.13	1.13
Calcium, %	0.96	0.96	0.96	0.75	0.75	0.75	0.66	0.66	0.66
Available phosphorus, %	0.48	0.48	0.48	0.44	0.44	0.44	0.40	0.40	0.40

[1]Diets used in this study included the following: 1) unmedicated corn-soybean meal (**SBM**) basal without SDP (Control diet); 2) unmedicated corn-SBM basal into which bacitracin methylene disalicylate (**BMD**) was added at 0.055 g/kg diet (BMD diet); and 3) SDP diet in which spray-dried plasma was incorporated into unmedicated corn-SBM basal at 3% level (i.e., 30 g/kg diet). Each of these 3 diets were separately formulated for the starter (D 1 to 14), Grower (D 15 to 28), and finisher (D 29 to 42) phases of broiler production cycle.

[2]Vitamin Premix, supplied per kilogram of diet: Vitamin A (6,600 IU), Vitamin D (1,980 IU), Vitamin E (33 IU), Vitamin B12 (0.02 mg), Biotin (0.13 mg), Menadione (1.98 mg), Thiamine (1.98 mg), Riboflavin (6.60 mg), d-Pantothenic Acid (11.0 mg), Vitamin B6 (3.96 mg), Niacin (55.0 mg), Folic Acid (1.1 mg).

[3]Mineral Premix, supplied per kilogram of diet: Manganese (Mn), 60 mg; Zinc (Zn), 60 mg; Iron (Fe), 40 mg; Copper (Cu), 5 mg; Iodine (I), 1.2 mg; Cobalt (Co), 0.5 mg.

[4]Experimental diets were analyzed for proximate nutrient composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

In each experiment, day-old chicks in treatments CON-SE, BMD-SE, and SDP-SE were inoculated with SE by orally gavaging 1 mL of inoculum / chick. On the other hand, each chick in CON, BMD, and SDP treatments was mock-challenged in a similar manner with 1 mL of sterile BPW.

Isolation and Enumeration of Salmonella *spp. From Ceca and Liver*

In Experiment 1, on d 3 and d 14 postchallenge (**PC**), 2 chicks were randomly taken from each pen (totaling 8 chicks per treatment) and euthanized by CO_2 asphyxiation. Cecal lobes from each bird were aseptically collected in a preweighted Whirl-Pak filter bag, weighed and processed for isolation of *Salmonella* as described above. Liver was also aseptically excised from each bird on d 3 and processed for isolation of *Salmonella* as previously done when confirming that ceca of chicks (day-old) arriving from hatchery were free of SE.

To enumerate SE in cecal samples, 25 mL sterile BPW was added into each Whirlpak filter bag, and the contents of each bag were homogenized in a Stomacher 80 Microbiomaster at medium speed (approx. 230 rpm) for 60 s. A 10-fold serial dilution of each sample was done in 10 mL BPW (i.e., serial 10-fold dilutions up to 10^6), and 100μL of each dilution was plated on XLT4 agar using spread-plating technique. The XLT4 plates were incubated at 37°C and incubated for 48 h. Next, the number of black presumptive SE colonies on XLT4 agar plates was then counted for each sample. SE concentration was expressed as $\log_{10}$ CFU/g ceca content.

In Experiment 2, on d 3, 7, 14, and 28, two chicks were randomly taken from each pen (totaling 8 chicks per treatment) and euthanized by CO_2 asphyxiation. The cecal lobes and liver were collected aseptically as described above. Ceca samples for d 3, 7, and 14 were subjected to *Salmonella* enumeration methods as described for Experiment 1. Because *salmonella* loads in d 3 liver and d 28 ceca samples were expected to be below the detection limit of our enumeration method, the samples were first subjected to enrichment in TT and RV broths, followed by detection of SE on XLT4 agar, and subsequent confirmation of suspect black colonies by biochemical tests (with TSI and LIA slants), and serological test as previously described.

Monitoring Chick Growth Performance

In Experiment 1, body weight (**BW**), body weight gain (**BWG**), and feed intake (**FI**) of chicks were recorded on d 7 and 14. From these data, feed conversion ratio (**FCR**) was calculated. Mortality was also recorded daily throughout the 14-day experiment. In Experiment 2, BW, BWG, FI, and FCR of chicks were recorded on d 7, 14, 28, and 42 for the evaluation of broiler growth performance. Flock uniformity was calculated on d 14 and 42 of experiment as a measure of body weight variation within a flock (Abbas et al., 2010). Flock uniformity was calculated as "% within ± 10% of BW mean" using the following equation (Jackson et al., 2004): uniformity = 100-[(standard deviation/mean) × 100]. Mortality was also recorded daily throughout the 42-d experiment

Statistical Analyses

Each experiment was a completely randomized design (**CRD**) with 6 treatments arranged in a 3 (dietary treatments − CON, BMD, SDP) × 2 (*Salmonella* challenge - non-challenged versus SE-challenged) factorial. However, because treatments CON, BMD, and SDP consisting of chicks that were not exposed to SE remained negative for *Salmonella* throughout Experiments 1 and 2, all data for cecal SE concentrations, d 28 SE prevalence ratio in ceca, and d 3 liver SE invasion were analyzed by one-way ANOVA as dependent variables (Proc ANOVA, SAS Institute, 2004). Significant differences among means were determined using the Tukey option of the general linear model (**GLM**) procedure as a post hoc test, and data are presented as means ± SEM. Statements of statistical significance were based upon $P < 0.05$.

On the other hand, all growth performance data for main effects (dietary treatments and *Salmonella*-challenge treatments) and interactions for both experiments were analyzed by ANOVA, using the PROC GLM procedure of SAS software (SAS Institute, Cary, NC). Data were presented as least squares means ± SEM. Significant differences among means were determined using the Tukey option of the general linear model (**GLM**) procedure as a post hoc test. Statements of statistical significance were based upon $P < 0.05$. For response criteria that had significant interaction between diet x challenge, data for all experimental treatments were presented in the tables of results.

RESULTS

Establishment of SE infection was confirmed on d 3 PC in both Experiments. The chicks in treatment groups exposed to SE had 1.16 to 5.68 log $_{10}$ CFU SE / g cecal content (Tables 2 and **3**), while the nonexposed groups (CON, BMD, and SDP) were negative for SE throughout the experiment (data not shown). On comparing Experiments 1 and 2 on d 3 PC, it was observed that cecal SE concentrations in challenged chicks in Experiment 1 (1.24−2.28 log $_{10}$ CFU / g cecal content; Table 2) was about half of the concentrations observed in Experiment 2 (3.39−5.68 log $_{10}$ CFU / g cecal content; Table 3).

Cecal Salmonella Concentration and Liver Invasion

In Experiment 1, chicks were reared on used litter. On d 3 PC, BMD-fed chicks had lower cecal SE concentration ($P < 0.05$) compared to CON-fed chicks (**Table 2**). A similar trend was observed for liver invasion on d 3.

Table 2. Effect of dietary spray-dried plasma on the concentration of *Salmonella* Enteritidis in ceca and liver of broiler chicks (Experiment 1).

Treatments[1]	Log$_{10}$ CFU / g cecal contents		Liver invasion ratio[3]
	Day 3 PC[2]	Day 14 PC	(Day 3 PC)
CON-SE	2.16 ± 0.22[a]	2.28 ± 0.29[a]	6/8[a]
BMD-SE	1.24 ± 0.24[b]	1.54 ± 0.43[b]	1/8[b]
SDP-SE	2.28 ± 0.27[a]	1.16 ± 0.03[b]	4/8[ab]
SEM	0.247	0.104	0.161
P-value	0.0131	0.0001	0.0390

[a,b]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.46 × 10^8 CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]PC, postchallenge.

[3]Liver invasion Ratio = number of birds whose liver(s) were positive for *Salmonella* Enteritidis / Total number of birds evaluated in each treatment category.

Table 3. Effect of dietary spray-dried plasma on the concentration of *Salmonella* Enteritidis in ceca and liver of broiler chickens (Experiment 2).

Treatment[1]	Log_{10} CFU / g cecal contents			Prevalence ratio[3] in ceca (Day 28 PC)	Liver invasion[4] ratio (Day 3 PC)
	Day 3 PC[2]	Day 7 PC	Day 14 PC		
CON-SE	5.68 ± 0.36^{a}	4.17 ± 0.18^{a}	2.48 ± 0.05^{a}	6/8	$6/8^{a}$
BMD-SE	3.39 ± 0.14^{b}	2.41 ± 0.12^{b}	1.53 ± 0.09^{b}	4/8	$1/8^{b}$
SDP-SE	3.58 ± 0.11^{b}	2.26 ± 0.09^{b}	1.06 ± 0.05^{c}	6/8	$5/8^{a}$
SEM	0.246	0.143	0.073	0.264	0.244
P-value	<0.0001	<0.0001	<0.0001	0.7479	0.0001

[a-c]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.48×10^{8} CFU *Salmonella* Enteritidis (SE) /mL at 1 d of age.

[2]PC, postchallenge.

[3]Prevalence Ratio (SPR) in ceca = number of birds whose ceca were positive for *Salmonella* Enteritidis / Total number of birds evaluated in each treatment category.

[4]Liver invasion Ratio = number of birds whose liver(s) were positive for *Salmonella* Enteritidis / Total number of birds evaluated in each treatment category.

However, by d 14, SDP-fed chicks also had lower cecal SE ($P < 0.05$) compared to CON-fed chicks. In Experiment 2. In Experiment 2, chicks were reared on fresh clean litter. On d 3 PC, BMD (3.39 $\log_{10}$ CFU / g cecal content) and SDP (3.58 $\log_{10}$ CFU / g cecal content) had similar ($P > 0.05$) cecal SE concentrations, and these values were lower ($P < 0.05$) than that of CON (5.68 $\log_{10}$ CFU / g cecal content; **Table 3**). A similar trend was observed on d 7. However, by d 14 PC, the mitigation efficiency of SDP against SE (1.06 $\log_{10}$ CFU / g cecal content) was superior ($P < 0.05$) to that of BMD (5.68 $\log_{10}$ CFU / g cecal content). Liver invasion ratio was lower ($P < 0.05$) for BMD-fed chicks compared to SDP- and CON-fed chicks (Table 3), as observed in Experiment 1. From these results, SDP showed at least similar efficacy to BMD in reducing cecal SE in broiler chicks during the first 2 wk of life.

Growth Performance, Flock Uniformity, and Mortality

Growth performance, mortality, and flock uniformity data for Experiments 1 and 2 are presented in Tables 4 to 8. In Experiment 1, birds were raised on used litter and differences were not observed among treatments for the parameters evaluated ($P > 0.05$; **Table 4**), and total mortality was 3.05%.

Table 4. Effect of dietary spray-dried plasma on growth performance of broiler chicks (day 1 to 14, Experiment 1).

Treatment[1]	Body weight (BW, kg/bird)[2]	Body weight gain (BWG, kg/bird)	Feed intake (FI, kg/bird)	FCR[3] (Kg:Kg)
Diet effect means				
CON	0.465	0.426	0.695	1.646
BMD	0.468	0.417	0.691	1.662
SDP	0.466	0.418	0.705	1.679
Pooled SEM	0.012	0.011	0.011	0.052
*Salmonella*challenge effect means				
Nonchallenge[4]	0.465	0.413	0.694	1.686
SE	0.468	0.427	0.700	1.639
Pooled SEM	0.010	0.009	0.009	0.043
Sources of variation	Probability			
Dietary treatment	NS[5]	NS	NS	NS
Challenge[1]	NS	NS	NS	NS
Diet x Challenge	NS	NS	NS	NS

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.46×10^{8} CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]Values are based only on weight of live birds.

[3]FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

[4]Non-challenge = pooled mean of treatments in which chicks were not challenged with *Salmonella* Enteritidis. These treatments are CON, BMD, and SDP.

[5]NS, not significant.

In Experiment 2, on d 7, chicks in BMD and SDP treatments had higher BW and BWG ($P < 0.05$) compared to chicks in all other treatments (Table 4). Feed intake was reduced by SE challenge ($P < 0.05$) and FCR was affected by dietary treatment. Specifically, SDP-fed chicks had superior FCR ($P < 0.05$) compared to chicks CON-fed chicks, while the FCR of BMD-fed chicks was in-between. Between d 8 and d 14, comparison of BWG revealed that only chicks in SDP-SE had higher BWG (0.325 Kg; $P < 0.05$) compared to its corresponding non-challenged treatment (SDP; 0.260 Kg). Feed intake and FCR was similar (FI = 0.389 to 0.438 Kg; FCR = 1.379 to 1.556) for all treatments ($P > 0.05$), except for CON-SE that had a higher FI (0.516 Kg; $P < 0.05$) and poorer FCR (2.048; $P < 0.05$). Between d 15 and d 28 of Experiment 2, BMD-fed chicks had higher BWG ($P < 0.05$) compared to CON- and SDP-fed chicks. Birds fed CON and BMD diets had higher FI ($P < 0.05$) compared to birds fed SDP diet. Among SE-exposed chicks, only BMD-SE treatment had superior FCR (1.001; $P < 0.05$) to CON-SE treatment (1.307). Between d 29 and d 42, SDP-fed birds had lower BW and BWG ($P < 0.05$) compared to CON- and BMD-fed birds. Furthermore, SE-exposed birds had higher BWG and FI ($P < 0.05$) compared to non-challenged birds. This could be due to compensatory growth as the birds recover from the SE-infection. With the exception of birds in SDP-SE treatment, FCR was similar for all treatments (1.425 to 1.515; $P > 0.05$). Birds in SDP-SE treatment had poorer FCR (1.759; $P < 0.05$) than all other treatments.

Evaluation of cumulative growth performance from d 1 to d 42 of experiment revealed that SE-exposed birds that were fed BMD diet had higher BWG (2.92 Kg; $P < 0.05$) compared to SE-exposed birds fed SDP diet (2.17 Kg), while the BWG of CON-SE birds was in-between. The FI of SDP-fed birds were similar ($P > 0.05$) to that of BMD-fed birds, but lower ($P < 0.05$) than that of CON-fed birds. The FI of SE-challenged birds was also higher ($P < 0.05$) that the FI of non-challenged birds. Among nonchallenged birds (i.e. CON, BMD, and SDP), FCR was similar ($P > 0.05$), while among SE-exposed chicks, FCR was best for BMD-SE treatment (1.311) and poorest for SDP-SE (1.606), with that of CON-SE in-between (1.581). Upon comparing the nonchallenged treatments (i.e., CON, BMD, and SDP) to their corresponding SE-exposed treatments, it was observed that CON (1.511) and BMD (1.442) had similar FCR values ($P > 0.05$) to CON-SE (1.581) and BMD-SE (1.311), respectively. On the other hand, the FCR of SDP (1.455) was superior ($P < 0.05$) to that of SDP-SE (1.606). This implied that while BMD and SDP are effective in reducing cecal SE concentration, the FCR of SDP-fed birds was compromised, while the FCR of BMD-fed birds were unaffected.

Flock uniformity and mortality was influence by SE-challenge throughout Experiment 2. Flock uniformity was lower for SE-challenged birds ($P < 0.05$) compared to nonchallenged birds on d 14 and 42. Total mortality was 7.5% and the higher mortality observed in SE-challenged birds (10.56%) compared to nonchallenged birds (4.45%) approached significance ($P = 0.0509$).

Salmonella challenge affected flock uniformity and percent mortality (Table 8). Compared to non-challenged birds, flock uniformity was lower ($P < 0.05$) for ST-challenged birds on d 14, and for both SE- and ST-challenged birds on d 42. Mortality was higher for ST-challenged birds (16.66%; $P < 0.05$) compared to NC birds (4.45%), with that of SE in-between (10.56%; Table 7).

DISCUSSION

Two experiments were conducted to determine the efficacy of porcine SDP supplementation at 30 g/kg diet and BMD antibiotic (at 0.055g/kg diet) in reducing cecal SE colonization in broiler chickens. In Experiments 1 and 2, chicks obtained from a commercial hatchery for experimentation were confirmed to be free of the nalidixic acid-resistant SE marker strain used in this study. Susceptibility of chicks to *Salmonella* colonization can be influenced by the level of pathogen exposure (infectious dose), competition with gut microflora for colonization sites, virulence of infecting *Salmonella* serovar (whether the strains carry genetic factors that facilitate attachment to the birds' gastrointestinal tracts or evade host defenses), integrity of intestinal epithelial barrier, age, and genetic predisposition of the bird (Bailey, 1988; Bailey, 1993; Carrasco et al., 2019).

In this study, SE infection was successfully established in the ceca of chicks in both Experiments 1 and 2. However, there were differences in the degree of colonization. Chicks in Experiment 1 had lower cecal SE concentrations (1.24−2.28 log $_{10}$ CFU / g cecal content; Table 2) compared to chicks in Experiment 2 (3.39−5.68 log $_{10}$ CFU / g cecal content; Table 3). This could be due to the differences in litter condition used during these experiments. Birds in Experiment 1 were reared on used litter, while those in Experiment 2 were reared on fresh clean litter. It has been established that a reciprocal relationship exists between cecal microbiota and litter microbiota, such that the level of *Faecalibacterium prausnitzii*, a commensal butyrate-producing species is increased in the cecum of chicks, while levels of halotolerant/alkaliphilic bacteria species are increased in the litter (Wang et al., 2016; Carrasco et al., 2019). Perhaps, an increased level of butyrate-producing bacteria (such as *Faecalibacterium prausnitzii*) in the ceca of chicks reared on used litter in Experiment 1 increased butyrate levels and decreased epithelial oxygenation, thereby reducing aerobic multiplication of cecal SE in these birds (Rivera-Chávez et al., 2016). The reverse was probably the case for chicks raised on clean litter in Experiment 2.

In this study, both BMD and SDP diets containing BMD antibiotic (at 0.055g/kg diet) and SDP (at 30 g/kg diet) respectively, were effective in reducing ceca *Salmonella* colonization during the first 2 wk of life, while only BMD diet reduced systemic invasion of the liver. The mitigating effect of BMD against SE was

Table 5. Effect of dietary spray-dried plasma on growth performance of broiler chicks from day 1 to 14 (Experiment 2).

	Day 1 to 7 (Parameters measured)[2]				Day 8 to 14 (Parameters measured)[2]			
Treatments[1]	Body weight (BW, kg/bird)[3]	Body weight gain (BWG, kg/bird)	Feed intake (FI, kg/bird)	FCR[4] (Kg:Kg)	BW (kg/bird)[3]	BWG (kg/bird)	FI (kg/bird)	FCR[4] (Kg:Kg)
CON	0.143[b]	0.102[b]	0.151[ab]	1.482[a]	0.409	0.266[ab]	0.413[b]	1.556[b]
BMD	0.178[a]	0.138[a]	0.169[a]	1.231[ab]	0.482	0.286[ab]	0.408[b]	1.429[b]
SDP	0.187[a]	0.147[a]	0.146[ab]	0.994[b]	0.444	0.260[b]	0.389[b]	1.496[b]
CON-SE	0.145[b]	0.101[b]	0.138[ab]	1.374[ab]	0.430	0.257[b]	0.516[a]	2.048[a]
BMD-SE	0.156[b]	0.112[b]	0.131[ab]	1.192[ab]	0.466	0.311[ab]	0.426[b]	1.379[b]
SDP-SE	0.149[b]	0.109[b]	0.124[b]	1.142[ab]	0.442	0.325[a]	0.438[b]	1.386[b]
P-value	<0.0001	<0.0001	0.0461	0.0224	0.1041	0.0105	0.0005	0.0003
Pooled SEM (24)	0.004	0.005	0.009	0.086	0.017	0.013	0.014	0.074
Diet Effect Means								
CON	0.144[b]	0.102[b]	0.144	1.428[a]	0.420[b]	0.262[b]	0.464[a]	1.802[a]
BMD	0.167[a]	0.125[a]	0.150	1.211[ab]	0.474[a]	0.298[a]	0.417[b]	1.404[b]
SDP	0.168[a]	0.128[a]	0.135	1.068[b]	0.443[ab]	0.293[ab]	0.414[b]	1.441[b]
Pooled SEM	0.003	0.004	0.006	0.061	0.012	0.009	0.010	0.052
*Salmonella*challenge effect means								
NC[5]	0.169[a]	0.129[a]	0.155[a]	1.235	0.445	0.271[b]	0.403[b]	1.494
SE	0.150[b]	0.107[b]	0.131[b]	1.236	0.446	0.298[b]	0.460[a]	1.604
Pooled SEM	0.003	0.003	0.005	0.050	0.010	0.007	0.008	0.043
Sources of variation	*Probability*							
Dietary treatment	0.0002	0.004	NS[6]	0.0043	0.0228	0.0284	0.0049	0.0003
Challenge[1]	0.0001	0.0002	0.0065	NS	NS	0.0228	0.0003	NS
Diet x Challenge	0.0020	0.0093	NS	NS	NS	0.0413	0.0268	0.0027

[a,b]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.48×10^8 CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]Values represent the mean of 4 replicate pens per treatment.

[3]Values are based only on weight of live birds.

[4]FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

[5]NC = non-challenged treatments. This represents pooled mean of treatments in which chicks were not challenged with *Salmonella* spp. These treatments are CON, BMD, and SDP.

[6]NS, not significant.

probably due to its ability to inhibit bacteria cell wall synthesis by preventing the dephosphorylation of C_{55}-isopropenyl pyrophosphate (Hutchings et al., 2019). On the other hand, SDP reduced cecal SE probably by enhancing gut mucosa barrier structure via decreasing SE-induced local inflammation (Perez-Bosque et al., 2016), and by inducing significant increase in the number of goblet cells in the intestinal epithelium, thereby possibly increasing mucin secretion (Liu et al., 2018; Jababu et al., 2020). It has been documented that increased intestinal mucin secretion prevents the attachment of pathogenic bacteria (Johansson and Hansson, 2016).

Infection of ceca by SE adversely affected the growth performance of SDP-fed birds. For instance, the superior FCR observed for SDP-SE birds on d 7 dwindled as they grew older (d 7 FCR = 1.142; d 14 = 1.386; d 28 = 1.251; d 42 = 1.759; Tables 5 to 8). In contrast, BMD-SE birds were able to maintain a superior FCR ($P < 0.05$; d 7 FCR = 1.131; d 14 = 1.379; d28 = 1.001; d 42 = 1.425; Tables 5 to 8) particularly between d 15 to d 42. The superior FCR observed for BMD-SE birds could be due to its modulation of cecal microbiota through promoting the growth of beneficial bacteria and/or inhibiting or eliminating pathogenic microorganisms. For instance, a recent study with turkeys revealed that dietary supplementation of BMD decreased the abundance of members of the phylum *Candidatus Saccharibacteria* (TM7), but increased the abundance of members of the *Lachnospiraceae* family (Johnson et al., 2019). An increase in members of *Lachnospiraceae* spp. has been reported in chickens that have improved feed efficiency (Stanley et al., 2015; De Cesare et al., 2017).

Furthermore, SE infection reduced bird uniformity and showed a propensity to increase mortality in Experiment 2 (Table 8). On d 42, nonchallenged chicks had a higher uniformity of 70.90% ($P < 0.05$) compared to SE-challenged chicks (52.72%). Although growth-promoting feed additives such as prophylactic antibiotics (Engster et al., 2002) and SDP (Bregendahl et al., 2005) have been shown to enhance broiler body weight uniformity, such beneficial effects were not observed in SE-exposed BMD- and SDP-fed birds in this study. This is a concern because improvement in flock body weight uniformity is one of the most important economic indicators in broiler production. Furthermore, a more uniform flock causes fewer disruptions for machinery during slaughter and downstream carcass processing (Engster et al., 2002). A positive correlation has been shown to exist between early growth rate and the uniformity of carcass weight at market (Leeson, 2016), thus emphasizing the importance mitigating *Salmonella* colonization in the intestine and ceca of poultry. In Experiment 2, total mortality was 7.5%, which is higher than the values

Table 6. Effect of dietary spray-dried plasma on growth performance of broilers from day 15 to 42 (Experiment 2).

	Day 15 to 28 (Parameters measured)[2]				Day 29 to 42 (Parameters measured)[2]			
Treatments[1]	Body weight (BW, kg/bird)[3]	Body weight gain (BWG, kg/bird)	Feed intake (FI, kg/bird)	FCR[4] (Kg:Kg)	BW (kg/bird)[3]	BWG (kg/bird)	FI (kg/bird)	FCR[4] (Kg:Kg)
CON	1.46	1.01^{ab}	1.25^{ab}	1.246^{ab}	2.53^{ab}	1.15^{ab}	1.72	1.500^{b}
BMD	1.60	1.10^{ab}	1.33^{ab}	1.206^{ab}	2.54^{ab}	1.09^{b}	1.56	1.430^{b}
SDP	1.46	1.00^{ab}	1.15^{ab}	1.161^{ab}	2.44^{ab}	1.04^{b}	1.54	1.480^{b}
CON-SE	1.48	1.03^{ab}	1.33^{a}	1.307^{a}	2.84^{ab}	1.33^{ab}	2.02	1.515^{b}
BMD-SE	1.55	1.27^{a}	1.24^{ab}	1.001^{b}	2.93^{a}	1.43^{a}	2.03	1.425^{b}
SDP-SE	1.36	0.89^{b}	1.11^{b}	1.251^{ab}	2.33^{b}	1.03^{b}	1.80	1.759^{a}
P-value	0.2046	0.0128	0.0273	0.0209	0.0159	0.0069	0.0190	< 0.0001
Pooled SEM (24)	0.065	0.058	0.046	0.053	0.112	0.069	0.103	0.023
Diet Effect Means								
CON	1.47	1.02^{b}	1.29^{a}	1.276^{a}	2.68^{a}	1.24^{a}	1.87	1.508^{b}
BMD	1.57	1.18^{a}	1.28^{a}	1.104^{b}	2.74^{a}	1.26^{a}	1.79	1.427^{c}
SDP	1.41	0.95^{b}	1.13^{b}	1.206^{ab}	2.38^{b}	1.04^{b}	1.67	1.620^{a}
Pooled SEM	0.046	0.041	0.033	0.037	0.079	0.049	0.073	0.016
*Salmonella*challenge effect means								
NC[5]	1.51	1.04	1.24	1.204	2.50	1.10^{b}	1.61^{b}	1.470^{b}
SE	1.46	1.06	1.23	1.186	2.70	1.26^{a}	1.95^{a}	1.566^{a}
Pooled SEM	0.037	0.033	0.027	0.030	0.065	0.040	0.060	0.013
Sources of variation	*Probability*							
Dietary treatment	NS[6]	0.0044	0.0069	0.0209	0.0178	0.0122	NS	< 0.0001
Challenge[1]	NS	NS	NS	NS	0.0502	0.0118	0.0017	0.0003
Diet x Challenge	NS	NS	NS	0.0302	NS	NS	NS	< 0.0001

[a,b]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.48×10^8 CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]Values represent the mean of 4 replicate pens per treatment.

[3]Values are based only on weight of live birds.

[4]FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

[5]NC = nonchallenged treatments. This represents pooled mean of treatments in which chicks were not challenged with *Salmonella* spp. These treatments are CON, BMD, and SDP.

[6]NS, not significant.

(4.5%−5%) reported by Dozier et al. (2017) for similar strain of birds at the end of a 49-d experiment, during which they fed conventional corn-soybean meal basal diet supplemented with graded levels of distillers dried grains with solubles. The higher mortality observed in this study was probably due to SE infection in the ceca of SE-exposed birds.

The SDP diet performed similarly to BMD up to 14 d, but only BMD continued to improve growth performance till the end of experiment. There appeared to be no further benefit of dietary SDP beyond d 14 with respect to cecal *Salmonella* concentration, and beyond d 28 with respect to growth performance. Accordingly, dietary SDP supplementation at 30 g/Kg diet showed similar mitigation potential to BMD in reducing cecal *Salmonella* spp. colonization only during the first 2 wk of life in broiler chicks. (Tables 2 and 3).

In summary, both BMD and SDP reduced cecal SE colonization in broiler chickens during the first 2 weeks of life. However, liver invasion results showed that only BMD restricted the systemic spread of SE in experimental birds ($P < 0.05$; Tables 2 and 3). Litter condition also seems to influence bird susceptibility to intestinal *Salmonella* colonization, with reduced SE colonization in birds reared on used litter. The SDP diet mitigated the adverse effect(s) of SE challenge on broiler growth performance up to 2 wk of age, while BMD was effective in improving BWG throughout the 42-d trial (Table 7). However, neither SDP nor BMD improved flock uniformity. It is suggested that a regimen of multiple bioactive growth-promoting feed additives should be utilized at different stages of the broiler production cycle. Herein, we propose dietary regimen in which SDP should be used as in-feed prophylactic growth promoter in starter diets during the first 2 to 3 wk of life, followed by replacement with BMD or non-antibiotic additive with equivalent potency in the grower and finisher diets. It was concluded that dietary SDP supplementation at 30 g/Kg diet showed similar mitigation potential to BMD in reducing cecal SE colonization only during the first 2 wk of life in broiler chicks.

This is the first definitive study documenting the efficacy of SDP to reduce cecal *Salmonella* spp. load in poultry. Further investigation is needed to determine the underlying molecular mechanisms by which SDP reduce intestinal *Salmonella* colonization in neonate poultry. Results from such investigations will likely unravel novel avenues that can be exploited for enhancing the efficacy of SDP as an alternative to antibiotic growth promoters in poultry production.

Table 7. Effect of dietary spray-dried plasma on cumulative growth performance of broilers from day 1 to 42 (Experiment 2).

	Parameters measured[2]		
Treatments[1]	Body weight gain (BWG, kg/bird)[3]	Feed intake (FI, kg/bird)	FCR[4] (Kg:Kg)
CON	2.35[b]	3.54[ab]	1.511[abc]
BMD	2.40[b]	3.46[ab]	1.442[dc]
SDP	2.23[b]	3.23[b]	1.455[bc]
CON-SE	2.54[ab]	4.00[a]	1.581[ab]
BMD-SE	2.92[a]	3.83[ab]	1.311[d]
SDP-SE	2.17[b]	3.48[ab]	1.606[a]
P-value	0.0030	0.0113	0.0001
Pooled SEM (24)	0.104	0.126	0.029
Diet effect means			
CON	2.44[ab]	3.77[a]	1.546[a]
BMD	2.66[a]	3.65[ab]	1.377[b]
SDP	2.20[b]	3.35[b]	1.531[a]
Pooled SEM	0.073	0.089	0.020
*Salmonella*challenge effect means			
NC[5]	2.33[b]	3.41[b]	1.470
SE	2.54[a]	3.77[a]	1.500
Pooled SEM	0.060	0.073	0.017
Sources of variation	*Probability*		
Dietary treatment	0.0028	0.0171	0.0001
Challenge[1]	0.0250	0.0045	NS
Diet x Challenge	0.0490	NS	0.0012

[a-d]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment **BMD** consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.48×10^8 CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]Values represent the mean of 4 replicate pens per treatment.

[3]Values are based only on weight of live birds.

[4]FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

[5]NC = non-challenged treatments. This represents pooled mean of treatments in which chicks were not challenged with *Salmonella* spp. These treatments are CON, BMD, and SDP. NS, not significant.

Table 8. Effect of dietary spray-dried plasma on broiler flock uniformity (Experiment 2).

Treatments[1]	Flock uniformity[2] day 14 (%)	Flock uniformity[2] day 42 (%)	Mortality (%)
Diet effect means			
CON	53.13	56.58	9.17
BMD	60.50	67.38	8.33
SDP	66.94	61.47	5.00
Pooled SEM	5.30	4.52	2.53
*Salmonella*challenge effect means			
NC[3]	71.98[a]	70.90[a]	4.45[b]
SE	48.41[b]	52.72[b]	10.56[ab]
Pooled SEM	4.33	3.69	2.07
Sources of variation	*Probability*		
Dietary treatment	NS[4]	NS	NS
Challenge[1]	0.0023	0.0045	0.0509
Diet x Challenge	NS	NS	NS

[a-c]Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

[1]Treatment CON consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment BMD consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g/kg diet; Treatment SDP consisted of chicks given unmedicated corn-SBM basal into which SDP was added at 30 g/kg diet; Treatments CON-SE, BMD-SE, and SDP-SE, consisted of chicks that were given diets similar to CON, BMD, and SDP, respectively, and were each inoculated with 7.48×10^8 CFU *Salmonella* Enteritidis /mL at 1 d of age.

[2]Values represent the mean of 4 replicate pens per treatment.

[3]NC = nonchallenged treatments. This represents pooled mean of treatments in which chicks were not challenged with *Salmonella* spp. These treatments are CON, BMD, and SDP.

[4]NS, not significant.

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DISCLOSURES

The authors declare no conflicts of interest.

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