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 log10 CFU / g and 3.58 log10 CFU / g, respectively), but these levels were lower (P < 0.05) than that of CON-fed chicks (5.68 log10 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), comingleing 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
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 has 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 nutritious and palatable feed additive which contain functional proteins and essential nutrients that include biologically active peptides (defensins, transferrins), immunoglobulins, 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 mucus 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 Biologicals, 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, 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.
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 CO2 asphyxiation. Cecal lobes from each bird were aseptically collected in a preweighed 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 isolation and enumeration methods as described for Experiment 1. Because salmonella concentrations 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 7 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, Cary, NC). 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 $P < 0.05$.

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).

| Treatments1 | Log$_{10}$ CFU / g cecal contents | Liver invasion ratio (Day 3 PC) |
|-------------|----------------------------------|--------------------------------|
|             | Day 3 PC2 | Day 14 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$^b$ | 1.16 ± 0.03$^b$ | 4/8$^{ab}$ |
| SEM         | 0.247     | 0.104     | 0.161                            |
| P-value     | 0.0131    | 0.0001    | 0.0390                           |

1$^a$bMean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

1$^a$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 $7.46 	imes 10^8$ CFU Salmonella Enteritidis /mL at 1 d of age.

2PC, postchallenge.

3Liver 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, 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 ($P > 0.05$); total mortality was 3.05%.

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

| Treatment | Log$_{10}$ CFU / g cecal contents | Prevalence ratio in ceca (Day 28 PC) | Liver invasion ratio (Day 3 PC) |
|-----------|-----------------------------------|-------------------------------------|-------------------------------|
| CON-SE | 5.68 ± 0.30$^a$ | 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 |
| $P$-value | <0.0001 | <0.0001 | <0.0001 |

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

1Treatment 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 $7.48 \times 10^8$ CFU *Salmonella* Enteritidis (SE) /mL at 1 d of age.

2PC, postchallenge.

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

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

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

| Treatment | Body weight (BW, kg/bird) | Body weight gain (BWG, kg/bird) | Feed intake (FI, kg/bird) | FCR$^3$ (Kg:Kg) |
|-----------|--------------------------|--------------------------------|--------------------------|---------------|
| 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 | NS | NS | NS | NS |
| Diet x Challenge | NS | NS | NS | NS |

1Treatment 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 $7.46 \times 10^8$ CFU *Salmonella* Enteritidis /mL at 1 d of age.

2Values are based only on weight of live birds.

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

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

5NS, 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 CON-fed chicks, while the FCR of BMD-fed chicks was lower for SE-challenged birds ($1.455$) was superior ($< P < 0.05$) compared to CON- and BMD-fed birds. Furthermore, SE-exposed birds 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 ($2.92$ Kg; $P < 0.05$) 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 that of CON- and BMD-fed birds. 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. Among SE-challenged treatment, 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.

**DISCUSSION**

Two experiments were conducted to determine the efficacy of porcine SDP supplementation at $30$ g/kg diet and BMD antibiotic (at $0.055$g/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 naldixic acid-resistant SE marker strain used in this study. Susceptibility of chicks to *Salmonella* colonization can be influenced by the level of pathogen exposure (infections 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 $0.055$g/kg diet) and SDP (at $30$ g/kg diet) respectively, were effective in reducing cecal *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
probably due to its ability to inhibit bacteria cell wall synthesis by preventing the dephosphorylation of C55-isopropenyl pyrophosphate (Hutchings et al., 2019). On the other hand, SDP reduced cecal SE probably by increasing mucin secretion (Liu et al., 2018; Jababu et al., 2020). It has been documented that dietary supplementation of BMD decreased the abundance of members of the phylum Candidatus Saccharibacteria (TMT), 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 5. Effect of dietary spray-dried plasma on growth performance of broiler chicks from day 1 to 14 (Experiment 2).

| Treatments | Body weight (BW, kg/bird) | Body weight gain (BWG, kg/bird) | Feed intake (FI, kg/bird) | FCR (Kg:Kg) | BW (kg/bird) | BWG (kg/bird) | FI (kg/bird) | FCR (Kg:Kg) |
|------------|--------------------------|-------------------------------|--------------------------|-------------|-------------|---------------|--------------|-------------|
| CON        | 0.143b                   | 0.102b                        | 0.151b                   | 1.425       | 0.409       | 0.266ab       | 0.413b       | 1.556b      |
| BMD        | 0.178                    | 0.138                         | 0.169                    | 1.231b      | 0.482       | 0.286ab       | 0.405b       | 1.429b      |
| SDP        | 0.187b                   | 0.147                         | 0.146b                   | 0.994b      | 0.444       | 0.260b        | 0.380b       | 1.496b      |
| CON-SE     | 0.145b                   | 0.101b                        | 0.138b                   | 1.374b      | 0.430       | 0.257b        | 0.516b       | 2.948b      |
| BMD-SE     | 0.156b                   | 0.112b                        | 0.131b                   | 1.192b      | 0.466       | 0.311b        | 0.426b       | 1.379b      |
| SDP-SE     | 0.140b                   | 0.109b                        | 0.124b                   | 1.142b      | 0.442       | 0.325a        | 0.438b       | 1.386b      |

Pooled SEM (24) 0.004 0.005 0.009 0.086 0.017 0.013 0.014 0.074

Day 1 to 7 (Parameters measured)^2

| Challenge | Body weight | Body weight gain | Feed intake | FCR |
|-----------|-------------|------------------|-------------|-----|
| NC        | 0.169a      | 0.129a           | 0.155a      | 1.235 |
| SE        | 0.150b      | 0.107b           | 0.131b      | 1.236 |
| Pooled SEM | 0.003      | 0.003            | 0.005       | 0.010 |

Day 8 to 14 (Parameters measured)^2

| Challenge | Body weight | Body weight gain | Feed intake | FCR |
|-----------|-------------|------------------|-------------|-----|
| NC        | 0.169^a     | 0.129^a          | 0.155^a     | 1.235^a |
| SE        | 0.150^b     | 0.107^b          | 0.131^b     | 1.236^b |
| Pooled SEM | 0.003      | 0.003            | 0.005       | 0.010 |

^a,bMean values bearing different superscript letters within a column are significantly different (P < 0.05).

^1Treatment 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 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.

^2Values are based only on weight of live birds.

^3Values are based only on weight of live birds.

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

^5NC = 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.

^6NS, not significant.
Table 6. Effect of dietary spray-dried plasma on growth performance of broilers from day 15 to 42 (Experiment 2).

| Treatments | Body weight (BW, kg/bird) | Body weight gain (BWG, kg/bird) | Feed intake (FI, kg/bird) | FCR (Kg:Kg) | BW (kg/bird) | BWG (kg/bird) | FCR (Kg:Kg) |
|------------|--------------------------|---------------------------------|--------------------------|-------------|-------------|-------------|-------------|
| CON        | 1.46                     | 1.01ab                           | 1.25ab                   | 1.246ab     | 2.53ab      | 1.15ab      | 1.72        | 1.50b       |
| BMD        | 1.60                     | 1.10ab                           | 1.33ab                   | 1.206b      | 2.54ab      | 1.09b       | 1.56        | 1.430b      |
| SDP        | 1.46                     | 1.00b                            | 1.15ab                   | 1.161b      | 2.44ab      | 1.04b       | 1.54        | 1.480b      |
| CON-SE     | 1.48                     | 1.03ab                           | 1.33a                    | 1.307a      | 2.84ab      | 1.33ab      | 2.02        | 1.515b      |
| BMD-SE     | 1.55                     | 1.27a                            | 1.24ab                   | 1.00b       | 2.93a       | 1.43a       | 2.03        | 1.425b      |
| SDP-SE     | 1.36                     | 0.89b                            | 1.11b                    | 1.251ab     | 2.33b       | 1.03b       | 1.80        | 1.759a      |
| P-value    | 0.2046                   | 0.0128                           | 0.0273                   | 0.0299      | 0.0159      | 0.0069      | 0.0190      | <0.0001     |
| Pooled SEM (24) | 0.0065 | 0.0058                           | 0.0046                   | 0.0053      | 0.112       | 0.069       | 0.103       | 0.023       |

Diet Effect Means

| Source of variation | Probability |
|---------------------|-------------|
| Dietary treatment   |             |
| Challenge           |             |
| Diet x Challenge    |             |

| Treatments | Dietary treatment | Challenge | Diet x Challenge |
|------------|-------------------|-----------|------------------|
| CON        | NS                | NS        | NS               |
| BMD        | NS                | NS        | NS               |
| SDP        | NS                | NS        | NS               |
| CON-SE     | NS                | NS        | NS               |
| BMD-SE     | NS                | NS        | NS               |
| SDP-SE     | NS                | NS        | NS               |

| Pooled SEM (24) | 0.0065 | 0.0058 | 0.0046 | 0.0053 |

| Probability | 0.0001 |

a,bValues bearing different superscript letters within a column are significantly different (P < 0.05).

1Treatment 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.

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

3Values are based only on weight of live birds.

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

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

6NS, 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).

| Treatments 1 | Body weight gain (BWG, kg/bird) | Feed intake (FI, kg/bird) | FCR 4 (Kg:Kg) |
|--------------|--------------------------------|--------------------------|----------------|
| CON          | 2.35 b                         | 3.54 ab                  | 1.51 abc       |
| BMD          | 2.40 b                         | 3.46 ab                  | 1.44 bc        |
| SDP          | 2.23 b                         | 3.23 b                   | 1.45 bc        |
| CON-SE       | 2.54 ab                        | 4.00 a                   | 1.58 ab        |
| BMD-SE       | 2.92 a                         | 3.83 ab                  | 1.31 d         |
| SDP-SE       | 2.17 b                         | 3.48 ab                  | 1.606a         |

*P*-value 0.0030 0.0113 0.0001
Pooled SEM (24) 0.104 0.126 0.029

Diet effect means
- CON: 2.44ab 3.77a 1.546a
- BMD: 2.66a 3.65ab 1.377b
- SDP: 2.20b 3.35b 1.531a
- Pooled SEM: 0.073 0.089 0.020

Salmonella challenge effect means
- NC: 2.33b 3.41b 1.470
- SE: 2.54a 3.77a 1.500
- Pooled SEM: 0.060 0.073 0.017

Sources of variation
- Dietary treatment: NS
- Challenge: 0.0028 0.0171 0.0001
- Diet x Challenge: NS

*a-d Mean values bearing different superscript letters within a column are significantly different (P < 0.05).
1Treatment 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 × 10⁸ CFU Salmonella Enteritidis/mL at 1 d of age.
2Values represent the mean of 4 replicate pens per treatment.
3NC = 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.
4FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

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: 71.98a 70.90a 4.45b
- SE: 48.41b 52.72b 10.56ab
- Pooled SEM: 4.33 3.69 2.07

Sources of variation
- Dietary treatment: NS
- Challenge: 0.0023 0.0045 0.0509
- Diet x Challenge: NS

*a-b Mean values bearing different superscript letters within a column are significantly different (P < 0.05).
1Treatment 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 × 10⁸ CFU Salmonella Enteritidis/mL at 1 d of age.
2Values represent the mean of 4 replicate pens per treatment.
3NC = 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.
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DISCLOSURES

The authors declare no conflicts of interest.

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