Evaluating the effects of a dietary synbiotic or synbiotic plus enhanced organic acid on broiler performance and cecal and carcass *Salmonella* load

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ABSTRACT Several feed additives such as synbiotics and organic acids may be viable options for controlling *Salmonella* in poultry. This experiment was conducted to study the effects of synbiotic product or synbiotic plus enhanced organic acid program on broiler performance, intestinal histomorphology, and cecal and carcass *Salmonella* load. A total of 648 day-of-hatch Cobb 700 male broiler chicks were randomly allocated to one of 4 dietary treatments: basal control diet (**CON**), CON diet supplemented with a synbiotic (PoultryStar; 500 g/MT; PS), CON diet supplemented with PS in the starter phase and enhanced organic acid (Biotronic PX Top3 US; 500g/MT; BPX) in the grower and finisher phase (PS1+BPX2), and the CON diet supplemented with PS in the starter and grower phase and BPX in the finisher phase (PS2+BPX1). No differences in overall BW or BWG ($P > 0.05$) were observed among PS, PS1+BPX2, and PS2+BPX1; however, BW was consistently greater ($P < 0.05$) in PS, PS1+BPX2, and PS2+BPX1 compared with CON on d 14 28, 35, and 42. On d 1 to 14 and d 1 to 28, PS and PS2+BPX1 improved FCR compared to CON ($P < 0.05$); PS1+BPX2 had intermediate results. No differences ($P > 0.05$) in overall FI were observed among dietary treatments, although PS1+BP2 and PS2+BPX1 increased FI numerically compared to CON and PS. Both PS1+BPX2 and PS2+BPX1 had reduced carcass *Salmonella* load by 1.6 and 1.4 log units, respectively, compared with CON ($P < 0.05$); PS had intermediate results. Birds fed PS1+BPX2 and PS2+BPX1 reduced the percentage of postchilled carcasses that tested positive for *Salmonella* by 72% and 57%, respectively, compared to CON, while PS had intermediate results with a 43% reduction. This experiment demonstrated that dietary supplementation with synbiotic or synbiotic plus organic acid can be used as a potential tool to improve growth performance and reduce carcass *Salmonella* in broilers.

Key words: synbiotic, organic acid, performance, histomorphology, *Salmonella*

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INTRODUCTION

Salmonella continues to be monitored in poultry products as it is one of the main human pathogens contributing to foodborne illness. Members of the *Salmonella* species that are public health concerns are associated with significant morbidity and mortality in those infected with the pathogen (Foley et al., 2008). There are over 40,000 reported cases of *Salmonella* infection in humans and 400 deaths reported annually in the United States (Fàbrega and Vila, 2013). The incidence rate of *Salmonella* increased from 14.53 cases per 100,000 in 2005 to 17.55 in 2010, and 15.19 in 2013; yearly variation ranged from 0.5% to 16.8% and the average annual percent change was an increase of 1.3% from 2005 to 2013 (Johnson et al., 2014). The risks of acquiring this disease are greatly influenced by the prevalence of *Salmonella* in poultry and poultry products; these risks are influenced by sources of these agents for poultry and modes of spread within flocks and during processing of poultry, propagation of salmonellae on farms and within processing plant, and survival of these pathogens on farms and during processing (Pires et al., 2014; Wideman et al., 2016). As part of its responsibility for ensuring the safety, wholesomeness, and accurate labeling of meat, poultry, and pasteurized egg products, the U.S. Department of Agriculture (**USDA**) and Food Safety and Inspection Service (**FSIS**) tests products for the presence of *Salmonella* (White et al., 2007). Three FSIS regulatory programs include testing for *Salmonella* in meat, poultry, and egg products: the Pathogen Reduction

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Hazard Analysis and Critical Control Point (**PR-HACCP**) verification program, the ready-to-eat (**RTE**) meat and poultry products program, and the pasteurized egg products program.

The reduced usage of antibiotics at the subtherapeutic level has increased the need for alternatives to assist in managing food safety risk and growth performance. Several feed additives such as synbiotics and organic acids may be viable options for controlling *Salmonella* in poultry. Synbiotics are additives that combine the use of probiotics and prebiotics such that they act synergistically. Probiotics are defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Borchers et al., 2009; Fuller, 2012). Probiotics have been shown to improve the balance of intestinal microbiota, reduce the population of pathogen microorganisms, stimulate the immune system, and enhance nutrient availability (Toms and Powrie, 2001; Khan and Naz, 2013). Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial microbiota in the digestive system (Manning and Gibson, 2004). Prebiotics have been shown to increase weight gain and carcass weight, improve digestibility, reduce *Salmonella* load, and improve food safety (Londero et al., 2011; Cengiz et al., 2012). Supplementation with synbiotics has been shown to significantly improve body weight, average daily gain, feed efficiency, and carcass yield compared to the controls or probiotic-fed broilers (Awad et al., 2009). Synbiotics were also shown to beneficially alter the intestinal microbiota composition and increase both villi height and crypt depth in the intestinal mucosa (Jung et al., 2008; Awad et al., 2009).

Dietary organic acids and their salts are generally regarded as safe (**GRAS**) and have been approved by most regulatory agencies to be used as a feed additive in animal production. Organic acids used in food production can be described chemically as either simple monocarboxylic acids (e.g., formic, acetic, propionic, and butyric acid) or carboxylic acids bearing a hydroxyl group (e.g., lactic, malic, tartaric, and citric acid) (Dibner and Buttin, 2002). Organic acids are considered to affect microbial activity by 2 primary mechanisms: by cytoplasmic acidification with subsequent uncoupling of energy production and regulation, and by accumulation of the dissociated acid anion to toxic levels (Taylor et al., 2012). The organic acids in nondissociated form can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria (Dhawale, 2005). In addition to their antimicrobial activity, organic acids reduce the pH of digesta, increase pancreatic secretions, and have trophic effects on the mucosa of the gastrointestinal tract (Dibner and Buttin, 2002). Acidification with various organic acids has been reported to reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall (Langhout, 2000), increase nutrient digestibility by elevating protein and dry matter retention, thus improving mineral absorption and phosphorous utilization (Rafacz-Livingston et al., 2005; Islam, 2012), improve gut health through direct effects on epithelial cells (Langhout, 2000), and improve growth performance (Biggs and Parsons, 2008; Panda et al., 2009; Samanta et al., 2010).

The objective of the current experiment was to evaluate a synbiotic and an enhanced organic acid on broiler performance, intestinal histomorphology, and cecal and carcass *Salmonella* load.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee: Texas A&M University (**TAMU**) Institutional Animal Care and Use Committee (AUP #2018-0181) and was consistent with the Guide for the Care and Use of Agricultural Animals in Research and Teaching guidelines (FASS, 2010).

Experimental Design

A total of 648 Cobb 700 male broiler chicks were obtained on day-of-hatch from a commercial hatchery. Birds were randomly assigned to 36 pens (0.91 m x 1.83 m) with 18 birds per pen and 9 pens per treatment, so that each pen contained birds of approximately equal initial BW. Pens were allocated to treatments in a randomized complete block design. Each pen was lined with used pine shavings as bedding material and equipped with one bell feeder and nipple drinking system. Birds were allowed ab libitum access to feed and water. Birds were housed in an environmentally controlled tunnel ventilated broiler house, building temperature was maintained at 31°C on d 1 to 7, reduced to 29°C on d 8 to 14, and then allowed to decrease 2.8°C each week until ambient temperature was reached. Birds were provided with a lighting regime of 22L:2D from 1 to 14 d of age, and 20L:4D from 15 to 42 d of age.

Experimental Diets

Diets were corn and soybean meal based and formulated to meet or exceed NRC (1994) recommendations for nutrients and energy. Analyzed nutrient content of the experimental diet is presented in (Table 1). Birds were fed phased diets in crumble form during starter (d 1−14) and pelleted form during grower (d 15−28), and finisher (d 29−42) phases. Four dietary treatments were used, as follows a control diet (NC) and 3 synbiotic or synbiotic plus enhanced organic acid. The NC was formulated to meet nutritional requirements. Other treatments were CON diet supplemented with a synbiotic (PoultryStar; 500g/MT; PS), CON diet supplemented with PS in the starter phase with enhanced organic acid (Biotronic PX Top3 US; 500g/MT; BPX) in the grower and finisher phase (PS1+BPX2), and the CON diet supplemented with PS in the starter and grower phase with BPX in the finisher phase (PS2

Table 1. Analyzed nutrient content of diets fed to broilers.[1]

Analyzed nutrient	Starter (D 1−14)	Grower (D 15−28)	Finisher (D 29−42)
Moisture (%)	9.91	11.31	11.78
Dry matter (%)	90.09	88.69	88.22
Protein (crude) (%)	21.90	20.20	18.90
Fat (crude) (%)	3.87	4.97	6.22
Fiber (acid detergent) (%)	2.60	2.90	1.90
Ash (%)	5.54	4.71	4.52
Metabolizable energy (Mcal/lbs)	1.38	1.40	1.44
Sulfur (total, %)	0.29	0.26	0.23
Phosphorus (total, %)	0.82	0.75	0.70
Potassium (total, %)	1.13	1.01	0.92
Magnesium (total, %)	0.19	0.17	0.15
Calcium (total, %)	1.22	0.85	0.86
Sodium (total, %)	0.11	0.12	0.16
Iron (total, ppm)	398.00	374.00	354.00
Manganese (total, ppm)	124.00	112.00	103.00
Copper (total, ppm)	37.90	14.20	19.80
Zinc (total, ppm)	114.00	109.00	105.00

[1]Analyzed nutrient package conducted by Midwest Laboratories, Inc., Omaha, NE.Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.Vitamin premix added at this rate yields 8,818 IU vitamin A, 3,086 IU vitamin D3, 37 IU vitamin E, 0.0132 mg B12, 4.676 mg riboflavin, 36.74 mg niacin, 16.17 mg d-pantothenic acid, 382.14 mg choline, 1.18 mg menadione, 1.4 mg folic acid, 5.74 mg pyridoxine, 2.35 mg thiamine, 0.44 mg biotin per kg diet. The carrier is ground rice hulls.

+BPX1). Diets were corn and soybean meal based. Pelleting temperature was maintained at 70°C.

Growth Performance

Mortalities were collected, recorded, and weighed daily. All birds and feed were weighed per pen on d 14, 28, and 42 (at the end of each dietary phase) for the determination of body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), and calculation of feed conversion ratio (**FCR**).

Histomorphological Measurements

On d 21 and d 42, a 1 cm-long section of ileum from the midway point between Meckel's diverticulum and the ileocecal junction was collected from 8 birds per treatment (1 bird per replicate). Ileal segments were rinsed with phosphate-buffered saline (Cat. #97063-6581, VWR, Radnor, PA) and stored in 30 mL of 10% neutral buffered formalin (Cat. #89370-094, VWR, Radnor, PA) at room temperature. Samples were sent to Histo-Scientific Research Laboratories (Mt. Jackson, VA) to be processed and stained with Periodic Acid-Schiff in combination with Alcian Blue. The mounted and stained ileum sections were then analyzed at 4x magnification using a Nikon Eclipse Ci-L microscope (Nikon Corporation, Tokyo, Japan). The accompanying Elements software package was used to measure villus height, crypt depth, and villus/crypt ratio from 6 villi per sample.

Microbial Analysis

On d 42, 8 birds per treatment were processed and rinstate samples were collected postchilling for analysis of *Salmonella*. Postchilled carcasses were placed in plastic bags (Cat. #89085-532, VWR, Radnor, PA) with 225 mL of buffered peptone water (Cat. #89407-426, Hardy Diagnostics, Santa Maria, CA). Carcasses were shaken by hand for 1 min to ensure consistent agitation. Following agitation, a bottom corner of the rinse bag was cut with scissors and 30 mL of the solution was decanted into a prelabeled conical tube (Cat. #89039-656, VWR, Radnor, PA). Samples were kept on ice during transport to the laboratory. In addition, ceca samples were collected from the same birds harvested for rinsate samples during processing. Enumeration of *Salmonella* from the ceca contents and postchilling rinsate samples was determined by performing a 10-fold dilution series, aliquots from each sample were transferred to xylose lysine tergitol 4 (XLT4) agar (Cat. # 89407-184, Hardy Diagnostics, Santa Maria, CA), incubated for 24 h at 42°C. Following incubation, colony-forming units (**CFU**) for *Salmonella* were calculated using a log scale.

Statistical Analysis

Statistical analyses were conducted with the Statistical Analysis Software (SAS, SAS Institute, Cary, NC) to determine if variables differed between treatment groups. The feed intake, feed conversion ratio, body weight, body weight gain, histomorphological parameters and *Salmonella* data were compared between groups using the GLIMMIX procedure of SAS. Main effects of treatment and day were used to analyze histomorphological measurements as well as their interaction. Goblet cell count data were analyzed using a Poisson distribution and estimates calculated using the ilink function. Distribution of positive samples for *Salmonella* was analyzed using Chi-squared frequency plot. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

Growth Performance

The effect of dietary treatment on feed consumption, body weight, body weight gain, and feed conversion are presented in (Table 2). Throughout the duration of the trial, no differences in overall BW or BWG ($P > 0.05$) were observed among PS, PS1+BPX2, and PS2+BPX1; however, BW was consistently greater ($P < 0.05$) in PS, PS1+BPX2, and PS2+BPX1 compared with CON on d 14 28, 35, and 42. Similarly, BWG was greater ($P < 0.05$) in PS, PS1+BPX2, and PS2+BPX1 compared with CON on d 14, 28, and 35. No differences ($P > 0.05$) in overall BWG was observed among dietary treatments; however, PS, PS1+BPX2, and PS2+BPX1 were numerically greater than CON by 3.80, 4.60, and 3.90%, respectively. No differences ($P > 0.05$) in FI were

Table 2. Effect of synbiotic alone, or in combination with an enhanced organic acid on growth performance in broilers.[1]

	BW, kg	FI, kg	BWG, kg	FCR
D 0				
CON	0.40	-	-	-
PS	0.40	-	-	-
PS1+BPX2	0.40	-	-	-
PS2+BPX1	0.40	-	-	-
Pooled SEM	0.000	-	-	-
P-value	0.271	-	-	-
D 1−7				
CON	0.161^{b}	0.172	0.116^{b}	1.491
PS	0.166^{ab}	0.177	0.121^{ab}	1.462
PS1+BPX2	0.166^{ab}	0.181	0.121^{ab}	1.499
PS2+BPX1	0.171^{a}	0.182	0.125^{a}	1.458
Pooled SEM	0.002	0.003	0.002	0.019
P-value	0.026	0.105	0.039	0.362
D 1−14				
CON	0.445^{b}	0.493^{b}	0.399^{b}	1.235^{a}
PS	0.475^{a}	0.512^{ab}	0.428^{a}	1.198^{b}
PS1+BPX2	0.477^{a}	0.523^{a}	0.430^{a}	1.216^{ab}
PS2+BPX1	0.482^{a}	0.524^{a}	0.434^{a}	1.207^{b}
Pooled SEM	0.006	0.007	0.006	0.009
P-value	<0.001	0.019	<0.001	0.029
D 1−21				
CON	0.877	1.108^{b}	0.831	1.334
PS	0.913	1.150^{a}	0.866	1.330
PS1+BPX2	0.903	1.159^{a}	0.856	1.354
PS2+BPX1	0.910	1.173^{a}	0.862	1.359
Pooled SEM	0.012	0.018	0.012	0.013
P-value	0.189	0.079	0.191	0.276
D 1−28				
CON	1.577^{b}	2.005^{b}	1.529^{b}	1.311^{a}
PS	1.658^{a}	2.056^{ab}	1.609^{a}	1.278^{b}
PS1+BPX2	1.647^{a}	2.089^{a}	1.599^{a}	1.307^{a}
PS2+BPX1	1.640^{a}	2.099^{a}	1.591^{a}	1.320^{a}
Pooled SEM	0.020	0.024	0.020	0.009
P-value	0.036	0.043	0.039	0.023
D 1−35				
CON	2.295^{b}	3.287^{b}	2.247^{b}	1.463
PS	2.398^{a}	3.367^{ab}	2.348^{a}	1.434
PS1+BPX2	2.378^{a}	3.444^{a}	2.330^{a}	1.480
PS2+BPX1	2.390^{a}	3.397^{a}	2.340^{a}	1.452
Pooled SEM	0.025	0.034	0.026	0.013
P-value	0.026	0.024	0.032	0.099
D 1−42				
CON	2.934^{b}	4.797	2.886	1.665
PS	3.051^{a}	4.794	3.000	1.599
PS1+BPX2	3.074^{a}	4.975	3.025	1.645
PS2+BPX1	3.053^{a}	4.875	3.003	1.623
Pooled SEM	0.037	0.059	0.037	0.020
P-value	0.049	0.128	0.055	0.124

[1]All performance data is corrected for mortality.

[a-e]Means within column with different superscripts differ at $P < 0.05$. Treatments: CON (Control), PS (PS fed d0-42), PS1+BPX2 (PS fed d0-14, BPX fed d15-42), PS2+BPX1 (PS fed d0-28, BPX fed d29-42).

observed among PS, PS1+BPX2, and PS2+BPX1. Diets containing PS1+BPX1 and PS2+BPX1 increased ($P < 0.05$) FI compared with CON at d 1−14, 1−21, 1−28, and 1−35. No differences ($P > 0.05$) in overall FI was observed among dietary treatments, although PS1 +BP2 and PS2+BPX1 increased FI numerically compared to CON and PS. Birds fed PS and PS2+BX1 improved ($P < 0.05$) FCR compared with CON through 14 d of age. Additionally, birds fed PS improved ($P < 0.05$) FCR compared with CON, PS1+BPX2, and PS2+BPX1 through 28 d of age. No differences ($P > 0.05$) in overall FCR was observed among dietary treatments, however PS improved overall FCR numerically compared to CON, PS1+BPX2, and PS2+BPX1 by 7, 5, and 3 points, respectively.

Histomorphological Measurements

The effect of dietary treatment on villus height, crypt depth, villus height: crypt depth ratio (V/C), goblet cell count, and goblet cell density for dietary treatments are presented in (Table 3). No was no interaction of treatment and day on villus height, crypt depth, or V/C ratio ($P > 0.05$) on d 21 or d 42; however, villus height increased from d 21 to d 42 ($P < 0.05$) vs. crypt depth was decreased ($P < 0.05$). On d 21, PS1+BPX2 and CON increased ($P < 0.05$) goblet cell count when compared with PS and PS2+BPX1. On d 42, PS2+BPX1 increased ($P < 0.05$) goblet cell count when compared with all dietary treatments; PS1+BPX2 and CON increased ($P < 0.05$) goblet cell count when compared with PS. On d 21, CON increased ($P < 0.05$) goblet cell density when compared with PS; PS1+BPX2 and PS2 +BPX1 had intermediate results. On d 42, PS2+BPX1 increased ($P < 0.05$) goblet cell density when compared with all dietary treatments.

Microbial Analysis

The effect of dietary treatment on *Salmonella* prevalence and enumeration are presented in (Table 4). No differences in *Salmonella* prevalence or enumeration

Table 3. Effect of a synbiotic alone or in combination with an enhanced organic acid on histomorphological parameters in broilers.

	Villus height, μm	Crypt depth, μm	V/C Ratio	Goblet cell count	Goblet cell density
D 21					
CON	741.56	227.81	3.51	141.96^{d}	19.18^{bc}
PS	771.45	237.14	3.45	127.06^{e}	16.52^{d}
PS1+BPX2	783.65	231.08	3.93	140.96^{d}	18.16^{bcd}
PS2+BPX1	700.88	218.55	3.37	119.9^{f}	17.63^{cd}
P-value (treatment)	0.025	0.129	0.912	<0.001	0.048
D 42					
CON	1036.25	220.35	5.33	202.83^{b}	19.57^{b}
PS	957.65	189.75	5.50	179.13^{c}	18.82^{bc}
PS1+BPX2	1047.10	221.68	5.06	206.96^{b}	19.56^{b}
PS2+BPX1	986.20	194.23	5.31	218.29^{a}	21.63^{a}
P-value (treatment)	<0.001	<0.001	<0.001	<0.001	<0.001
P-value (treatment x day)	0.113	0.138	0.153	<0.001	0.049
Pooled SEM	17.45	6.78	0.157	1.865	0.719

[a-f]Means within column with different superscripts differ at $P < 0.05$. Treatments: CON (Control), PS (PS fed d 0−42), PS1+BPX2 (PS fed d 0−14, BPX fed d 15−42), PS2+BPX1 (PS fed d 0−28, BPX fed d 29−42).

Table 4. Effect of synbiotic alone, or in combination with an enhanced organic acid on ceca and carcass *Salmonella* in broilers.[1]

Ceca	CFU, Log 10	Positive, %
CON	1.66	50
PS	1.32	38
PS1+BPX2	1.08	38
PS2+BPX1	1.32	38
P-value	0.95	0.94
Post-chilled Carcass	CFU, Log 10	Positive, %
CON	2.06[a]	88[a]
PS	1.29[ab]	50[ab]
PS1+BPX2	0.53[b]	25[b]
PS2+BPX1	0.73[b]	38[b]
P-value	0.03	0.03

[1]Data were analyzed using frequency plot/chi-squared.
[a-b]Means within column with different superscripts differ at $P < 0.05$. Treatments: CON (Control), PS (PS fed d 0−42), PS1+BPX2 (PS fed d 0−14, BPX fed d 15−42), PS2+BPX1 (PS fed d 0−28, BPX fed d 29−42).

($P > 0.05$) from ceca samples were observed between dietary treatments. Postchilled carcass rinsate samples from birds fed PS1+BPX2 and PS2+BPX1 decreased ($P < 0.05$) *Salmonella* load by 1.6 and 1.4 log units respectively, compared to CON, while PS had intermediate results with a 0.8 log unit reduction. The distribution of percent of rinsate samples for *Salmonella* was influenced ($P < 0.05$) by treatment; birds fed PS1+BPX2 and PS2+BPX1 reduced the percentage of postchilled carcasses that tested positive for *Salmonella* by 72% and 57%, respectively, compared to CON, while PS had intermediate results with a 43% reduction.

DISCUSSION

Improvement in growth performance in broilers fed synbiotics is thought to be based on the concept that a mixture of probiotics and prebiotics beneficially affect the host by improving the survival and implantation of probiotic organisms and by selectively promoting growth or metabolism of beneficial bacteria in the gastrointestinal tract (Manning and Gibson, 2004; Awad et al., 2009; Mookiah et al., 2014). In the present study, the beneficial effects of a synbiotic on broiler growth performance parameters including BW, BWG, and FCR are in agreement with previous studies (Awad et al., 2009; Mousavi et al., 2015; Salag et al., 2018). Birds fed PS, PS1+BPX2, and PS2+BPX1 increased BW compared with CON throughout the study. A similar trend was observed for BWG, as PS, PS1+BPX2, and PS2+BPX1 increased BWG compared with CON on d 14, 28, and 35. Feed conversion was improved during the starter phase by PS (1.198) and PS2+BPX1 (1.207) compared with CON (1.235). Birds fed PS improved FCR compared with PS1+BPX2 (1.307) and PS2+BPX1 (1.320) during the grower phase. Although no differences in overall FCR were observed, birds fed PS (1.599) improved overall FCR compared to PS1+BPX2 (1.645) and PS2+BPX1 (1.623). The synbiotic (PS) improved broiler growth performance when compared with the un-supplemented diet, specifically BW and FCR. Feed conversion was improved in diets supplemented with the enhanced organic acid (BPX) compared to PS alone. Longer feeding of BPX resulted in the greatest overall FCR; however, longer feeding of BPX had a positive benefit on intestinal morphology and reduced *Salmonella* incidence compared to PS alone. In a study conducted by Awad et al. (2009), the inclusion of a synbiotic increased body weight and average daily gain, and improved feed conversion compared with the control and probiotic-fed broilers. These results are also consistent with the findings of Salag et al., (2018) , who reported the supplementation of a synbiotic and organic acid improved cumulative FCR when compared to birds fed an un-supplemented diet. The results from this study indicate the presence of a synbiotic in the diet through all phases of growth may be beneficial for improving growth performance in broilers.

Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). The villus crypt is considered as the "villus factory" and deeper crypts indicate faster tissue turnover to permit the renewal of the villus as needed in response to normal sloughing or inflammation from pathogens (Yason and Summers, 1987). A shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased gastrointestinal secretion, and lower performance (Xu et al., 2003); whereas increases in the villus height and villus height: crypt depth ratio are directly correlated with increased epithelial cell turnover (Fan et al., 2002). At the base of the crypts, goblet cells develop, which are located over the entire gastrointestinal tract. These cells secrete glycoproteins of a high molecular mass called mucins, the primary function of goblet cells is to secrete mucin and create a protective mucus layer. In the present study, no differences in villus height, crypt depth, or V/C ratio was observed among dietary treatments. On d 21, PS1+BPX2 increased goblet cell count compared with PS and PS2+BPX1. On d 42, PS2+BPX1 increased goblet cell count compared with all dietary treatments; PS1+BPX2 increased goblet cell count compared with PS. On d42, PS2+BPX1 increased goblet cell density when compared with all dietary treatments. In a study conducted by Awad et al. (2010), supplementation of broilers with a synbiotic increased the villus height and V/C ratio compared to the control. Garcia et al. (2007) and Senkoylu et al. (2007) reported increased villus height and surface area with formic acid (up to 1% of the diet) or the combination of formic and propionic acid (0.3% of the diet). These results are not in agreement with the present study. The addition of a synbiotic or synbiotic plus enhanced organic acid did not alter villus height, crypt depth, or V/C ratio; however, the addition of an enhanced organic acid did appear to have an effect on goblet cell count and density. Diets supplemented with BPX increased goblet cell count compared with PS alone at both d 21 and d 42. Goblet cell density was numerically increased in diets supplemented with BPX compared to PS alone on d 21. Similar results were observed on d 42, as PS2+BPX1 increased goblet cell density

compared with PS and PS1+BPX2. Kum et al. (2010) reported goblet cell frequency in the small intestine was significantly increased in birds fed diets supplemented with organic acids compare with the control. Mucin glycoproteins, synthesized and secreted by the goblet cells distributed along the villi play a key role in the intestinal epithelium function (Uni et al., 2003). The intestinal layer that is synthesized and secreted by goblet cells protects the brush border is and acts as the first line of defense against enteric pathogens by decreasing their adherence to the intestinal mucosa. These results indicate synbiotic plus enhanced organic acid supplementation exhibit some benefits on intestinal histology by promoting cell differentiation into goblet cells, which may reduce *Salmonella* incidence.

Enteric pathogens such as *Salmonella* are a major source of morbidity and mortality throughout the world (Foley et al., 2013). *Salmonella* infection in humans, known as salmonellosis, can cause symptoms such as diarrhea, fever, vomiting, and in some cases even death (Crum-Cianflone, 2008). *Salmonella enterica* is one of the most important food-borne pathogens and it typically acquired through the consumption of contaminated products of animal origin, such as poultry or eggs (Morris et al., 2011; Pires et al., 2014). In the present study, no differences in *Salmonella* prevalence or enumeration from ceca samples were observed among dietary treatments. Although no significant differences in *Salmonella* prevalence were detected, birds fed PS, PS1+BPX2, and PS2+BPX1 numerically reduced the percentage of ceca that tested positive for *Salmonella* compared to CON. Similar results were observed by Van Immerseel et al. (2004), the use of microencapsulated formic and acetic acids in the feed of chicks challenged individually at 5 d of age decreased the recovery of *Salmonella* Enteritidis in the ceca by 2.2 log units when sampled 3 d after challenge. Postchilled carcass rinsate samples from birds fed PS1 +BPX2 and PS2+BPX1 decreased *Salmonella* load by 1.6 and 1.4 log units respectively, compared to CON, while PS had intermediate results with a 0.8 log unit reduction. Similarly, birds fed PS1+BPX2 and PS2 +BPX1 reduced the percentage of postchilled carcasses that tested positive for *Salmonella* by 72% and 57% respectively, compared to CON, while PS alone had intermediate results with a 43% reduction. The addition of organic acids (acetic, formic, or lactic) as an alimentary tract clean-out has been used to decrease the recovery of *Salmonella* Typhimurium in the broiler's crop (3% positive with organic acids vs. 17% control) and also lead to a lower *Salmonella* prevalence on prechilled carcasses (15% positive with lactic acid vs. 31% control; Byrd et al., 2001). Dietary supplementation with organic acids is associated with reductions in bacteria, especially species which are acid-intolerant, such as *Escherichia coli, Salmonella*, and *Campylobacter* (Dibner and Buttin, 2002). Diets supplemented with organic acids may affect the microbiota of the intestinal tract through targeting the bacterial cytoplasmic membrane, thus disrupting the metabolic and replication functions (Denyer and Stewart, 1998; Davidson et al., 2012). These results indicate supplementation with the evaluated synbitoic plus organic acid could be used as a potential tool to reduce *Salmonella* on postchilled carcasses.

In conclusion, the synbiotic (PS) improved FCR through d 28 compared with the synbiotic plus organic acid (PS1+BPX2 and PS2+BPX1). Although no significant differences in overall FCR were observed, birds fed synbiotic improved overall FCR compared to synbiotic plus organic acid. No effects of synbiotic or synbiotic plus organic acid on BW or BWG were found. Birds fed synbiotic plus organic acid (PS2+BPX1) increased goblet cell count and density compared with other dietary treatments. No effects of synbiotic or synbiotic plus organic acid on villus height, crypt depth, or V/C ratio were found. Both synbiotic plus organic acid groups (PS1 +BPX2 and PS2+BPX1) reduced *Salmonella* load by 1.6 and 1.4 log units compared to the control. Similarly, both synbitoic plus organic acid groups (PS1+BPX2 and PS2 +BPX1) reduced the percentage of postchilled carcasses that tested positive for *Salmonella* by 72% and 57% compared to the control. The results from this study indicate dietary supplementation with a synbiotic may be considered a tool to improve growth performance, and supplementation with a synbiotic and organic acid may be used to reduce carcass *Salmonella* in broilers.

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript

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DISCLOSURES

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