Bacitracin, *Bacillus subtilis*, and *Eimeria* spp. challenge exacerbates woody breast incidence and severity in broilers

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ABSTRACT Woody breast (**WB**) is a myopathy that is related to the increasing growth rate. Understanding the influence of management factors on WB formation and development is important to minimize WB. This study was conducted to define how management factors affect broiler growth performance, processing yield, and WB incidence. Ross × Ross 708 chicks were randomly assigned to a 3 (diet) × 2 (cocci challenge) × 2 (sex) factorial arrangement of treatments. The 3 dietary treatments were: control diet (corn-soybean meal basal diet), antibiotic diet (basal diet + 6.075 mg bacitracin /kg feed), and probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6/kg feed). Birds in cocci challenge treatments received 20 × live cocci vaccine on d 14. The hardness of breast muscle in live birds was determined by palpation and grouped into Normal, Slight, Moderate, and Severe categories. Across diet and sex treatments, the cocci challenge resulted in decreases in body weight (**BW**) on d 29 and 35 ($P < 0.0001$ and $= 0.032$) in body weight gain (**BWG**) from d 14 to 29 ($P < 0.0001$). However, an increase of BW occurred on d 35 ($P = 0.032$) and an increase of BWG occurred from d 29 to 35 and d 35 to 43 ($P = 0.0001$ and 0.002), and the cocci challenge increased WB incidence on d 29 ($P = 0.043$) and d 43 ($P = 0.013$). Across challenge and sex treatments, birds fed the antibiotic diet exhibited a higher growth rate (**GR**) than those fed the control or probiotic diet from d 0 to 14 ($P = 0.016$), but not after d 14 ($P > 0.05$). Across sex, the antibiotic and probiotic diets increased WB incidence for those birds that did not receive a cocci challenge on d 43 ($P = 0.040$). Across challenge and diet treatments, males exhibited a higher BW, BWG, and GR throughout all growth phases, and males showed a higher WB incidence on d 29, 35, and 43 ($P = 0.002$, $P < 0.0001$, and $P = 0.0002$, respectively). In conclusion, bacitracin and *Eimeria* spp. increased WB incidence, BW, and GR. However, *Bacillus subtilis* increased WB incidence in male broilers without affecting BW and GR.

Key words: antibiotics, probiotics, coccidiosis, myopathy, gut health

2022 Poultry Science 101:101512
https://doi.org/10.1016/j.psj.2021.101512

INTRODUCTION

The broiler industry achieves high production efficiency and meat yield through genetic selection, disease control, nutritional strategies, and environmental management. However, rapid growth rate and increased meat yield of broilers are also associated with increased incidence of abnormalities such as myodegeneration, lipidosis, fibrosis, and regenerative alterations in broiler breast muscle (Tijare et al., 2016). These abnormalities result in myopathies such as white striping and woody breast (**WB**). WB is an abnormal meat condition in which the breast meat exhibits hardness and pale color (Sihvo et al., 2014; Bowker et al., 2019). A 3-year study showed that the incidence of moderate and severe WB was 25 to 35% among high breast yield broilers (Mallmann, 2019). Severe WB meat is downgraded, which leads to a reduced market price and greater than 200 million dollars in loss per year in the United States (Kuttappan et al., 2016). In response to the high incidence of WB, the broiler industry is exploring new nutritional and managerial approaches to reduce or eliminate WB incidence without negatively affecting growth performance and processing yield.

Although the etiology for WB is still unknown, the results of molecular biology studies have shown that in comparison to normal chicken breast meat that changes

Received August 6, 2021.
Accepted September 22, 2021.

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in oxidative stress responses, metabolic biosynthesis, and inflammation-related pathways contribute to WB muscle (Mutryn et al., 2015; Abasht et al., 2016; Zambonelli et al., 2016). Research has primarily addressed different dietary programs that can regulate nutrition metabolism, reduce oxidative stress, and attenuate inflammatory reactions (Olivo et al., 2001; Bodle et al., 2018; Livingston et al., 2019; Butler et al., 2020). The effect of dietary treatments commonly occurs in the broiler gut, which is a functional organ responsible for nutrient absorption. Dietary treatments such as subtherapeutic doses of antibiotics or probiotics have been used to improve broiler gut health and prevent intestinal diseases (Lee et al., 2012; Wang et al., 2018).

Coccidiosis is an intestinal disease caused by *Eimeria* spp., which results in intestinal integrity disorder and disrupts the digestion and absorption of nutrients, decreases gut barrier function, and increases susceptibility to bacterial infections (Chapman, 2014). Infections due to *Eimeria* spp. have also been shown to induce inflammatory responses and accelerate oxidative stress (Sepp et al., 2012; Griss et al., 2019). Inflammation and oxidative stress are considered as the main factors that cause myodegeneration, fibrosis, and lipidosis in WB muscle (Petracci et al., 2019). However, the extent to which *Eimeria* spp. infection can affect WB development is still unknown. Lesion scoring is a procedure used to evaluate coccidiosis. More specifically, the zone of the intestine that is parasitized and the gross appearance of the lesion are two primary characteristics that are used to differentially identify *Eimeria* infections (Conway and McKenzie, 2007).

Antibiotics are used to promote gut health in the poultry industry by controlling infectious agents and regulating immune responses (Nayebpor et al., 2007; Giovagnoni et al., 2019). However, dietary antibiotics decrease both pathogenic and commensal bacteria in the chicken's gut (Knarreborg et al., 2002). Furthermore, the metabolic activity of microflora and induced oxidative stress alter the response to antibiotics (Reese et al., 2018). Polypeptide bacitracin is an antibiotic used for growth promotion and prevention of infectious diseases in broiler farms and its growth promotion and gut modulation functions have been well studied (Brennan et al., 2003; Crisol-Martinez et al., 2017; Diaz Carrasco et al., 2018). Probiotics are used as an antibiotic alternative to promote broiler gut health by modulating gut microbiota, reducing oxidative stress, and improving immune response (Karimi Torshizi et al., 2010; Cisek and Binek, 2014; Giannenas et al., 2014; Zhang et al., 2016; Almeida Paz et al., 2019). *Bacillus subtilis* is a probiotic that functions in gut microbiota modulation and oxidative stress reduction. However, its growth promotion function has inconsistent results (Jayaraman et al., 2017; Wang et al., 2018; Musa et al., 2019; Sokale et al., 2019; Whelan et al., 2019).

In this study, we hypothesize that *Eimeria* spp. challenge and antibiotic additives will disrupt gut health and broiler growth performance, while probiotics will improve gut health. Therefore, the objective is to study the effects of an *Eimeria* spp. challenge, along with the antibiotic bacitracin and the probiotic *Bacillus subtilis* on the presence of intestinal lesions, growth performance, and WB incidence for male and female broilers.

MATERIALS AND METHODS

Facility and Rearing

This experiment was conducted on the Poultry Science Research Unit at Mississippi State University. All procedures used were approved by the Institutional Animal Care and Use Committee of Mississippi State University. One-day-old Ross 708 chicks were obtained from a commercial hatchery. Chicks were vaccinated against Marek's disease, Newcastle disease, and Infectious Bronchitis at the hatchery, but not for coccidiosis. Blocks were designated by their locations within an environmentally controlled house with 12 floor pens per block. Chicks were feather-sexed upon arrival, and a total of 672 male and 672 female Ross × Ross 708 chicks were randomly allotted to blocks with a total of 96 floor pens with 14 chicks per pen (0.086 m^2/broiler). Pens were equipped with commercial tube feeders, nipple drinkers, and top-dressed with fresh pine shavings. Wood boards with a height of 20 cm were placed between pens to avoid the cross-contamination of litter. Standard lighting programs were followed: 24 h of light from d 0 to 1, a 23L: 1D photoperiod from d 1 to 6, and a 20L: 4D photoperiod from d 7 to 44. The environmental heating program followed the temperature recommendations for Ross broilers (Aviagen, 2018). The starter, grower, and finisher diets were fed from d 0 to 14, 15 to 28, and 29 to 44, respectively; feed and water were provided ad libitum throughout the trial. Feed, water, and house temperature were monitored at 8 am and 4 pm every day. Mortality and the weights of dead birds were recorded daily.

Diet and Formulation

Before formulating the diets, corn and soybean meal samples were analyzed for total and digestible amino acid (**AA**) and apparent metabolic energy (**AME**) contents using a Foss NIR (near infrared) system (XDS-XM-1100 series, Foss, Sweden) and a commercial database (Precise Nutrition Evaluation, Adisseo, Alpharetta, GA). Basal diets were formulated using Concepts 5 Formulation (Educational Version 8.01.01, Creative Formulation Concepts LLC, Annapolis, MD) and made to meet the nutritional requirements for Ross 708 as-hatched broilers (Aviagen, 2019) (Table 1). The additional phytase additive was used to replace phosphorus by 0.1% (Table 1). Antibiotic diets were basal diets supplemented with 50 g/ton (or 0.025 g/lb) bacitracin methylene disalicylate (**BMD**), which is equivalent to 6.075 milligrams bacitracin/kg of finished feed. Probiotic diets were basal diets that were supplemented with 2.2×10^8 CFU *Bacillus subtilis* PB6 (CLOSTAT)/kg of finished feed. *Bacillus subtilis* counts of finished feed

Table 1. Feed ingredients composition and nutrient contents of a control diet during starter (D 0 to 14), grower (D 14 to 28), and finisher (D 28 to 44) feeding phases.

Ingredients %	Starter D 0 to 14	Grower D 14 to 28	Finisher d 28 to 44
Yellow corn[1]	61.19	67.29	72.76
Soybean meal[1]	31.12	24.81	18.76
Soybean oil	2.65	3.2	3.90
Dicalcium phosphate	1.72	1.49	1.81
Limestone	1.61	1.51	1.10
Salt	0.33	0.33	0.33
Choline chloride	0.01	0.01	0.02
L-lysine HCl	0.48	0.49	0.50
DL-methionine	0.43	0.4	0.37
Premix[2]	0.25	0.25	0.25
L-threonine	0.20	0.19	0.18
Ronozyme[3]	0.02	0.02	0.02
Calculated composition			
Crude protein, %	19.69	17.33	15.03
Calcium, %	0.96	0.87	0.78
Available phosphorous, %	0.38	0.34	0.39
M.E. (kcal/kg)	2,998	3,100	3,120
Digestible Lys, %	1.28	1.15	1.02
Digestible Met, %	0.72	0.66	0.61
Digestible TSAA, %	0.95	0.87	0.80
Digestible Thr, %	0.86	0.77	0.68
Choline (ppm)	771.15	725.79	680.40
Sodium, %	0.16	0.16	0.16
Potassium, %	0.79	0.69	0.60
Chloride, %	0.20	0.20	0.20

Abbreviations: M.E., metabolizable energy; TSAA, total sulfur amino acid.

[1]Nutrient composition was analyzed before formulating the diets.

[2]Premix provided the following per kilogram of finished diet: retinyl acetate, 2.654 μg; cholecalciferol, 110 μg; DL-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; vitamin B12, 0.01 mg; folic acid, 0.6 μg; choline, 379 mg; D-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin, 33 mg; thiamine, 1.0 mg; D-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.1 mg.

[3]Ronozyme (DSM Nutritional Products, Pendergrass, GA) is the phytase provided to replace 0.1% of phosphorus.

were confirmed. No anticoccidial additive was added to diets. The equivalent amount of sand was replaced by additives to ensure that the dietary nutrition remains unchanged. The diets were either crumbled (starter phase) or pelleted (grower and finisher phases).

Experimental Treatments

A 3 (diet) × 2 (cocci challenge) × 2 (sex) factorial arrangement of treatments in a randomized complete block design was used for this trial. The 3 diets were the control diet, antibiotic diet, and probiotic diet. The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati,* and *E. tenella*) or the same amount of distilled water on d 14.

Intestine Lesion Scoring

One bird from each pen on d 28 and 2 birds from each pen on d 42 were euthanized using CO_2 and the small intestine was collected from each bird. Duodenum (**Duo**), jejunum (**Jej**), and ileum (**Ile**) tissues were inspected for lesions (Conway and McKenzie, 2007). Red lesions caused by *E. maxima* and white lesions caused by *E. acerivulina* in the Duo, Jej, and Ile were observed and recorded. A lesion score of 0 indicated no gross lesion; a score of 1 indicated 1 to 4 petechiae on serosa/cm^2; a score of 2 indicated 5 to 10 petechiae on serosa/cm^2; and a score of 3 indicated 11or more petechiae on serosa/cm^2.

Growth Performance

Body weight (**BW**) and feed weight were recorded on d 0, 14, 28, 35, and 42 on a pen basis. Feed intake (**FI**) was adjusted by removing the feed consumption of dead birds. Body weight gain (**BWG**) and mortality corrected feed conversion ratio (**FCR**) were determined. Growth rate (**GR**) was calculated using the following equation:

$$Growth\ Rate\ (GR)\% = \frac{BW_{end} - BW_{initial}}{BW_{initial}} \times 100$$

WB Palpation

WB scoring in live birds was determined by hand palpation on d 28, 35, and 41. The procedure was developed by Tijare et al. (2016). The scores consisted of 4 categories, with 0 indicating normal breast muscle; 1 indicating slight WB with small areas of hardness on the breast muscle; 2 indicating moderate WB muscle in which half of the breast muscle was hard; and 3 indicating severe WB muscle with all of the breast muscle hard.

Processing

On d 44, 5 birds were randomly chosen for processing from each replicate pen. This included 480 birds in total from 12 treatments, with 8 replicates/treatment, and with 5 birds/replicate. Sixteen hours before processing, feed, and water were withdrawn from the birds. Birds were randomly selected for processing and deboning at the Mississippi State University Poultry Processing Facility. All birds were electrically stunned by manually placing the birds heads in a saturated saline bath (11.5 volts, <0.5 mA AC-DC current for 3 s). The shackle line speed was set at approximately 22 broilers per min. Unilateral neck cutting was manually performed immediately after stunning, and bleeding lasted for 140 s. Upon completion of exsanguination, the broilers were scalded at 53.3°C for 191 s, picked for 35 s using a rotary drum picker (Baader-Johnson, Kansas City, KS), and then mechanically eviscerated. Carcasses and fat pads were weighed after processing. Carcasses were chilled in 4°C water chilling tank for 4 h prior to manual deboning. After deboning, boneless and skinless breast muscle was palpated and scored using the same score system for WB scoring in live birds. Wing, drumstick, thigh, boneless and skinless breast, and tender were weighed after deboning.

Data Analysis

A randomized complete block design was used in this study. A three-way factorial arrangement of treatments was used to test for the main effects of diet, cocci challenge, and sex, as well as their interactive effects after d 14. Data collected before d 14 were analyzed using a 2-way factorial arrangement of treatments (diet, sex, as well as their interaction effects, since the cocci challenge was not imposed in that phase). Each of the 8 blocks in the housing facility served as a unit of replication. Each pen in each block was randomly assigned one of the 12 treatments. The categorical data of the intestinal lesion scores were analyzed using logistic regression, and treatment proportions were generated and separated using LSMEANS procedure with the ILINK option. The PROC GLM procedure (SAS Institute Inc., Cary, NC) was used to determine the main and interactive treatment effects on growth performance, processing yield, and WB incidence, with $P \leq 0.05$ used to denote significant differences among treatments. Fisher's protected least significant difference test was conducted to separate treatment means when main effects or interactive effects were significant. The correlation between WB and other measured variables were analyzed using Spearman's rank correlation test.

RESULTS

Lesion Score

In challenged birds across sex on d 28, the antibiotic diet resulted in a lower lesion proportion for Duo white lesions as compared to birds fed the control diet ($P = 0.0495$; Figure 1A). Furthermore, when averaged over sex and diet, the cocci challenge resulted in a greater Jej red lesion proportion on samples collected on d 28 ($P = 0.0003$; Figure 1B). In contrast, nonchallenged broilers exhibited a higher Jej red lesion proportion on samples collected on d 42 ($P = 0.0369$; Figure 2).

Growth Performance

Mortality Across challenge and diet treatments, female birds exhibited a higher mortality in comparison to

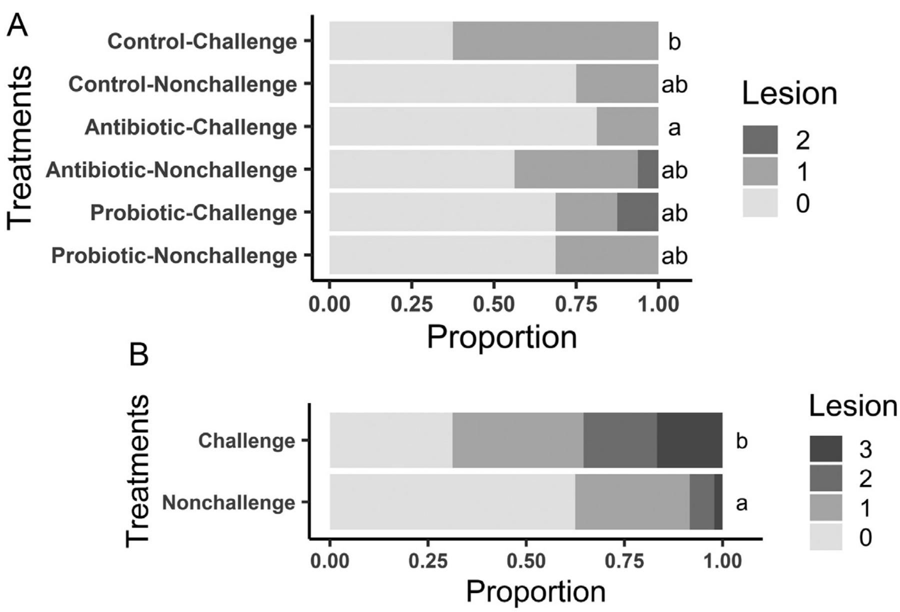

Figure 1. Lesion score probability of broiler intestine due to dietary additive and cocci challenge on duodenum white lesion (A, n = 16) and due to cocci challenge on jejunum red lesion (B, n = 48) on d 28. Lesion score = 0 indicating no gross lesion; score 1 indicating 1 to 4 petechiae on serosa/cm^2; score 2 indicating 5 to 10 petechiae on serosa/cm^2; score 3 indicating 11 to numerous petechiae on serosa/cm^2. a,b: Bars not sharing a common letter are different ($P < 0.05$).

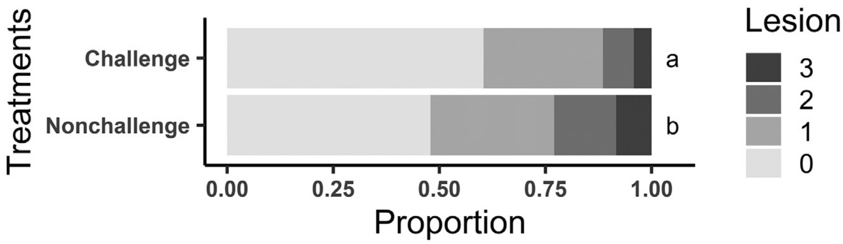

Figure 2. Lesion score probability of broiler intestine due to and cocci challenge on jejunum red lesion on d 42 (n = 48). Lesion score = 0 indicating no gross lesion; score 1 indicating 1 to 4 petechiae on serosa/cm^2; score 2 indicating 5 to 10 petechiae on serosa/cm^2; score 3 indicating 11 to numerous petechiae on serosa/cm^2. a,b: Bars not sharing a common letter are different ($P < 0.05$).

males from d 0 to 14 ($P = 0.023$; Table 2). No other differences existed among treatments after d 14 ($P > 0.05$) with respect to mortality.

BW There were no significant interaction effects between treatments on BW (Table 3). On d 29 and d 35, birds that were fed the antibiotic diet exhibited a higher BW as compared to birds that were fed the control and probiotic diet ($P = 0.004$ and $P = 0.003$, respectively). On d 29 and d 35, challenged birds exhibited a lower BW as compared to nonchallenged birds ($P < 0.0001$ and $P = 0.032$, respectively). However, diet or challenge did not affect BW on d 43 ($P = 0.052$). Compared to females, male birds exhibited a lower BW on d 14 ($P = 0.009$) but a higher BW from d 29 until d 43 (For all $P < 0.0001$).

BWG Similar to BW, there were no significant interaction effects between treatments for BWG (for all $P > 0.05$; Table 3). The antibiotic diet significantly increased BWG from d 0 to 14 as compared to the probiotic diet ($P = 0.007$), and from d 14 to 29 the antibiotic diet increased BWG when compared to both the control and probiotic diets ($P = 0.013$). The cocci challenge decreased BWG from d 14 to 29 ($P < 0.0001$) but increased BWG from d 29 to 35 ($P = 0.0001$) and d 35 to 43 ($P = 0.002$). Females exhibited a higher BWG than males from d 0 to 14 ($P = 0.004$), but males exhibited a higher BWG than females after d 14 (for all $P < 0.0001$).

FI Similar to BWG, there were no significant interactions between treatments for FI (Table 4). The antibiotic diet increased FI from d 0 to 14 as compared to the control and probiotic diets ($P = 0.003$), and from d 14 to 29 the antibiotic diet increased FI when compared to the probiotic diet ($P = 0.045$). The cocci challenge decreased FI from d 14 to 29 ($P = 0.003$), However, FI of birds in the challenged group increased after d 29 ($P = 0.001$, $P < 0.001$, and $P = 0.006$, respectively). Male birds always exhibited a higher FI than females after d 14 (for all $P < 0.0001$).

FCR There were also no significant treatment interactions for FCR except for a challenge × sex interaction (Table 5). The cocci challenge increased FCR from d 14 to 29 ($P < 0.0001$). Male birds exhibited a higher FCR than females from d 0 to 14 ($P = 0.004$), but a lower FCR than females from d 14 to 29 and d 35 to 43 ($P = 0.003$ and 0.0001, respectively). From d 29 to 35, the cocci

Table 2. Effects of dietary additive and cocci challenge on mortality (%) of male and female broilers.

Diet[1]	Challenge[2]	Sex	d 0 to 14	d 14 to 29	d 29 to 35	d 35 to 43	d 0 to 43
Control			0.223	0.240	0.260	0	0.670
Antibiotic			0.893	0.240	0.521	0.260	1.786
Probiotic			0	0.721	0.781	0.284	1.563
SEM[3]			0.2775	0.4340	0.4239	0.2239	0.5263
	Challenge		0.298	0.160	0.174	0.174	0.744
	Nonchallenge		0.446	0.641	0.868	0.189	1.935
	SEM[4]		0.2266	0.2467	0.3461	0.1828	0.4297
		Male	0[b]	0.481	0.694	0.363	1.339
		Female	0.744[a]	0.321	0.347	0	1.339
		SEM[5]	0.2266	0.2467	0.3461	0.1828	0.4297
P-value							
	Diet		0.067	0.434	0.687	0.611	0.290
	Challenge		0.644	0.172	0.160	0.952	0.054
	Sex		0.023	0.647	0.480	0.164	1.000
	Diet × Challenge		0.807	0.086	0.882	0.234	0.462
	Diet × Sex		0.067	0.434	0.882	0.611	0.290
	Challenge × Sex		0.644	0.172	0.160	0.952	0.146
	Diet × Challenge × Sex		0.807	1.000	0.419	0.234	0.290

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2 × 10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

Table 3. Effects of dietary additive and cocci challenge on body weight (BW) and body weight gain (BWG) of male and female broilers.

Treatment			BW (g)					BWG (g)			
Diet[1]	Challenge[2]	Sex	D 0	D 14	D 29	D 35	D 43	D 0 to 14	D 14 to 29	D29 to 35	D35 to 43[1]
Control			44.3	381[ab]	1,351[b]	1,843[b]	2,622	337[ab]	970[b]	492	778
Antibiotic			44.2	389[a]	1,383[a]	1,888[a]	2,661	345[a]	995[a]	504	773
Probiotic			44.1	375[b]	1,342[b]	1,840[b]	2,600	329[b]	964[b]	501	759
SEM[3]			0.15	3.3	9.0	10.8	17.6	3.3	7.5	5.4	14.3
	Challenge		44.3	384	1,332[b]	1,843[b]	2,640	341	947[b]	512[a]	796[a]
	Nonchallenge		44.1	378	1,386[a]	1,871[a]	2,616	333	1,006[a]	487[b]	744[b]
	SEM[4]		0.13	2.7	7.4	8.8	14.4	2.7	6.1	4.4	11.7
		Male	44.3	376[b]	1,405[a]	1,947[a]	2,801[a]	331[b]	1,026[a]	544[a]	854[a]
		Female	44.0	387[a]	1,313[b]	1,767[b]	2,453[b]	343[a]	926[b]	453[b]	686[b]
		SEM[5]	0.13	2.7	7.4	8.8	14.4	2.7	6.1	4.4	11.7
P-value											
Diet			0.591	0.013	0.004	0.003	0.052	0.007	0.013	0.270	0.617
Challenge			0.456	0.075	<00001	0.032	0.244	0.053	<0.0001	0.0001	0.002
Sex			0.085	0.009	<0.0001	<.0001	<0.0001	0.004	<0.0001	<0.0001	<0.0001
Diet × Challenge			0.954	0.727	0.657	0.523	0.906	0.695	0.748	0.717	0.857
Diet × Sex			0.161	0.167	0.321	0.244	0.573	0.21	0.208	0.839	0.665
Challenge × Sex			0.330	0.107	0.068	0.4	0.716	0.08	0.087	0.095	0.9
Diet × Challenge × Sex			0.554	0.768	0.841	0.639	0.696	0.634	0.863	0.325	0.22

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati,* and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

Table 4. Effects of dietary additive and cocci challenge on feed intake (FI) of male and female broilers.

Diet[1]	Challenge[2]	Sex	D 0 to 14	D 14 to 29	D 29 to 35	D 35 to 43	D 0 to 43
Control			459[b]	1,629[ab]	906	1,509	4,503
Antibiotic			470[a]	1,657[a]	917	1,522	4,565
Probiotic			453[b]	1,622[b]	907	1,504	4,487
SEM[3]			3.4	10.3	6.1	13.3	23.6
	Challenge		464	1,618[b]	922[a]	1,552[a]	4,557[a]
	Nonchallenge		457	1,654[a]	897[b]	1,471[b]	4,480[b]
	SEM[4]		2.8	8.4	5.0	10.9	19.3
		Male	458	1,708[a]	979[a]	1,641[a]	4,786[a]
		Female	464	1,564[b]	840[b]	1,382[b]	4,250[b]
		SEM[5]	2.8	8.4	5.0	10.9	19.3
P-value							
Diet			0.003	0.045	0.411	0.625	0.052
Challenge			0.083	0.003	0.001	<0.0001	0.006
Sex			0.154	<0.0001	<0.0001	<0.0001	<0.0001
Diet × Challenge			0.396	0.641	0.482	0.313	0.431
Diet × Sex			0.467	0.376	0.369	0.685	0.448
Challenge × Sex			0.308	0.055	0.8	0.581	0.219
Diet × Challenge × Sex			0.437	0.706	0.483	0.725	0.92

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati,* and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

challenge decreased the FCR of female birds ($P = 0.009$) but did not affect the FCR of male birds ($P > 0.05$).

GR Similar to BWG, there were no significant treatment interactions for GR. The cocci challenge lowered GR (BWG/initial BW) from d 14 to 29 ($P < 0.0001$; Table 6). Antibiotic supplementation improved GR as compared to probiotic supplementation only during the earlier age period from d 0 to 14 ($P = 0.016$) but not after d 14 ($P > 0.05$).

Processing Yield

There were no interaction effects of diet, challenge, and sex on processing data (for all $P > 0.05$; Tables 7 and 8). There was no significant difference in the live weight of processed birds due to diet or challenge ($P = 0.091$ and $P = 0.155$, respectively; Table 7). Furthermore, the effects of dietary antibiotic and probiotic supplementation on processing weights were not

Table 5. Effects of dietary additive and cocci challenge on feed conversion ratio (FCR) of male and female broilers.

Diet[1]	Challenge[2]	Sex	D 0 to 14	D 14 to 29	D 29 to 35	D 35 to 43
Control			1.350	1.655	1.850	1.903
Antibiotic			1.349	1.656	1.824	1.941
Probiotic			1.362	1.654	1.820	1.957
SEM[3]			0.0079	0.0101	0.0132	0.0189
	Challenge		1.350	1.692[a]	1.805	1.912
	Nonchallenge		1.358	1.618[b]	1.857	1.955
	SEM[4]		0.0064	0.0082	0.0107	0.0154
		Male	1.368[a]	1.637[b]	1.804	1.890[b]
		Female	1.340[b]	1.673[a]	1.859	1.977[a]
		SEM[5]	0.0064	0.0082	0.0107	0.0154
	Challenge	Male	1.356	1.670	1.798[b]	1.871
	Challenge	Female	1.344	1.715	1.813[b]	1.953
	Nonchallenge	Male	1.379	1.604	1.809[b]	1.909
	Nonchallenge	Female	1.336	1.632	1.906[a]	2.001
	SEM[6]		0.0091	0.0117	0.0152	0.0218
P-value						
Diet			0.445	0.992	0.235	0.116
Challenge			0.395	<0.0001	0.001	0.053
Sex			0.004	0.003	0.001	0.0001
Diet × Challenge			0.689	0.717	0.838	0.683
Diet × Sex			0.357	0.24	0.878	0.436
Challenge × Sex			0.09	0.467	0.009	0.814
Diet × Challenge × Sex			0.922	0.089	0.179	0.375

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.
[6]SEM, Standard error of mean for n = 24.

Table 6. Effects of dietary additive and cocci challenge on growth rate (GR) of male and female broilers.

Diet[1]	Challenge[2]	Sex	D 0 to 14	D 14 to 29	D 29 to 35	D 35 to 43
Control			760[b]	255	36.41	42.21
Antibiotic			781[a]	257	36.50	40.88
Probiotic			748[b]	259	37.41	41.22
SEM[3]			8.0	2.7	0.436	0.834
	Challenge		770	247[b]	38.39	43.14[a]
	Nonchallenge		756	267[a]	35.15	39.74[b]
	SEM[4]		6.5	2.2	0.356	0.681
		Male	748[b]	274[a]	38.88	43.97[a]
		Female	778[a]	240[b]	34.66	38.91[b]
		SEM[5]	6.5	2.2	0.356	0.681
	Challenge	Male	761	263	39.79[a]	45.38
	Challenge	Female	780	230	36.99[b]	40.91
	Nonchallenge	Male	734	285	37.98[b]	42.56
	Nonchallenge	Female	777	250	32.32[c]	36.92
	SEM[6]		9.2	3.1	0.503	0.963
P-value						
Diet			0.016	0.601	0.206	0.504
Challenge			0.116	<0.0001	<0.0001	0.001
Sex			0.001	<0.0001	<0.0001	<0.0001
Diet × Challenge			0.787	0.933	0.929	0.773
Diet × Sex			0.502	0.317	0.884	0.580
Challenge × Sex			0.195	0.605	0.006	0.547
Diet × Challenge × Sex			0.49	0.514	0.511	0.152

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.
[6]SEM, Standard error of mean for n = 24.

Table 7. Effects of dietary additive and cocci challenge on processing weights (g) of male and female broilers at 44 days of age.

Diet[1]	Challenge[2]	Sex	Live Weight	Carcass	Fat pad	Wings	Breast	Drumstick	Thigh	Tender
Control			2,676	1,869	45.2	208.0	525.6	243.0	332.7	111.0
Antibiotic			2,730	1,907	46.8	212.0	544.5	246.0	337.9	111.4
Probiotic			2,692	1,880	46.1	209.6	538.4	242.8	331.1	112.2
SEM[3]			17.8	13.7	0.93	1.57	5.74	1.82	2.95	1.21
	Challenge		2,714	1,908[a]	46.7	212.1[a]	544.5[a]	245.9	337.2	112.4
	Nonchallenge		2,685	1,862[b]	45.34	207.7[b]	527.8[b]	242.0	330.6	110.6
	SEM[4]		14.6	11.2	0.757	1.29	4.69	1.48	2.41	0.99
		Male	2,890[a]	2,021[a]	47.4[a]	222.2[a]	583.4[a]	263.4[a]	355.7[a]	115.4[a]
		Female	2,508[b]	1,749[b]	44.7[b]	197.5[b]	488.9[b]	224.5[b]	312.1[b]	107.6[b]
		SEM[5]	14.6	11.2	0.76	1.29	4.69	1.48	2.41	0.99
P-value										
	Diet		0.091	0.129	0.466	0.191	0.060	0.388	0.237	0.754
	Challenge		0.155	0.004	0.221	0.015	0.012	0.061	0.052	0.184
	Sex		<0.0001	<0.0001	0.015	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Diet × Challenge		0.579	0.547	0.403	0.832	0.914	0.702	0.634	0.901
	Diet × Sex		0.760	0.811	0.898	0.316	0.999	0.515	0.806	0.318
	Challenge × Sex		0.240	0.200	0.194	0.173	0.089	0.415	0.615	0.114
	Diet × Challenge × Sex		0.880	0.920	0.472	0.439	0.539	0.265	0.364	0.530

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2 × 10[8] CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

significantly different (for all $P > 0.05$). However, the cocci challenge increased carcass, wings, and breast weights ($P = 0.004$, $P = 0.015$, and $P = 0.012$, respectively). Male birds always exhibited higher processing weights as compared to female birds (for all $P < 0.05$). The effects of dietary antibiotic and probiotic supplementation on relative processing weight were not significantly different (for all P > 0.05; Table 8). The cocci challenge increased relative carcass weight ($P = 0.001$), but not other relative processing weights (for all $P > 0.05$). Male birds exhibited higher breast and drumsticks relative weight ($P < 0.0001$ and $P = 0.013$, respectively) and lower relative fat pad, wings, and tender weights than female (for all $P < 0.0001$).

WB Incidence

A cumulative WB score of 0 + 1 indicates that breast quality is acceptable, and a cumulate score of 2 + 3 indicates that breast quality is unacceptable (Tables 9−12). On d 29, male birds exhibited a lower incidence of

Table 8. Effects of dietary additive and cocci challenge on processing yields (%) of male and female broilers at 44 days of age.

Diet[1]	Challenge[2]	Sex	Carcass	Fat pad	Wing	Breast	Drumstick	Thigh	Tender
Control			69.86	1.69	11.16	28.03	13.02	17.84	5.95
Antibiotic			69.82	1.72	11.16	28.46	12.93	17.75	5.86
Probiotic			69.80	1.71	11.19	28.56	12.94	17.64	5.99
SEM[3]			0.229	0.031	0.061	0.173	0.072	0.110	0.049
	Challenge		70.29[a]	1.73	11.15	28.43	12.91	17.70	5.91
	Nonchallenge		69.36[b]	1.69	11.19	28.27	13.02	17.78	5.96
	SEM[4]		0.187	0.026	0.050	0.141	0.059	0.090	0.040
		Male	69.93	1.64[b]	11.02[b]	28.80[a]	13.07[a]	17.62	5.71[b]
		Female	69.72	1.78[a]	11.32[a]	27.89[b]	12.86[b]	17.86	6.15[a]
		SEM[5]	0.187	0.026	0.050	0.141	0.059	0.090	0.040
P-value									
	Diet		0.985	0.853	0.935	0.076	0.591	0.455	0.130
	Challenge		0.001	0.324	0.581	0.411	0.180	0.564	0.412
	Sex		0.423	<0.0001	<0.0001	<0.0001	0.013	0.064	<0.0001
	Diet × Challenge		0.064	0.240	0.228	0.763	0.916	0.299	0.250
	Diet × Sex		0.993	0.853	0.339	0.723	0.308	0.927	0.118
	Challenge × Sex		0.856	0.069	0.794	0.270	0.666	0.419	0.270
	Diet × Challenge × Sex		0.994	0.349	0.491	0.051	0.234	0.305	0.346

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2 × 10[8] CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

Table 9. Effects of dietary additive and cocci challenge on woody breast incidence of male and female broilers at 29 days of age.

Diet[1]	Challenge[2]	Sex	Score 0	Score 1	Score 2	Score 3	Score 0+1	Score 2+3
Control			89.51	8.64	1.85	0	98.15	1.85
Antibiotic			86.93	12.52	0.54	0	99.46	0.54
Probiotic			89.25	9.45	1.30	0	98.70	1.30
SEM[3]			1.455	1.336	0.598	0	0.598	0.598
	Challenge		87.41	10.65	1.94[a]	0	98.06[b]	1.94[a]
	Nonchallenge		89.73	9.75	0.52[b]	0	99.48[a]	0.52[b]
	SEM[4]		1.188	1.091	0.488	0	0.488	0.488
		Male	85.53[b]	12.74[a]	1.74	0	98.26	1.74
		Female	91.60[a]	7.67[b]	0.73	0	99.27	0.73
		SEM[5]	1.188	1.091	0.488	0	0.488	0.488
P-value								
Diet			0.390	0.102	0.308	.	0.308	0.308
Challenge			0.171	0.561	0.043	.	0.043	0.043
Sex			0.001	0.002	0.147	.	0.147	0.147
Diet × Challenge			0.349	0.667	0.308	.	0.308	0.308
Diet × Sex			0.746	0.762	0.258	.	0.258	0.258
Challenge × Sex			0.104	0.178	0.340	.	0.340	0.340
Diet × Challenge × Sex			0.214	0.378	0.206	.	0.206	0.206

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.

normal breast (score 0) ($P = 0.001$) but a higher incidence of WB score 1 than females ($P = 0.001$ and 0.002, respectively, Table 9). The cocci challenge significantly increased incidence of WB score 2 and score 2 + 3, but decreased incidence of WB score 0 + 1 (for all $P = 0.043$). On d 35, cocci challenge decreased incidence of normal breast ($P = 0.004$) and increased incidence of WB score 1 only on male birds ($P = 0.041$; Table 10). Male birds exhibited a higher incidence of WB score 2 and score 2 + 3, but lower incidence of WB score 0 + 1 (for all $P < 0.0001$). On d 43, nonchallenged birds that were fed antibiotic and probiotic diets exhibited a lower

Table 10. Effects of dietary additive and cocci challenge on woody breast incidence of male and female broilers at 35 days of age.

Diet[1]	Challenge[2]	Sex	Score 0	Score 1	Score 2	Score 3	Score 0+1	Score 2+3
Control			82.17	14.68	3.15	0	96.85	3.15
Antibiotic			80.04	16.24	3.72	0	96.28	3.72
Probiotic			82.83	14.23	2.94	0	97.06	2.94
SEM[3]			1.652	1.586	0.936	0	0.936	0.936
	Challenge		78.85	17.50	3.65	0	96.35	3.65
	Nonchallenge		84.51	12.59	2.89	0	97.11	2.89
	SEM[4]		1.348	1.295	0.765	0	0.765	0.765
		Male	77.55	16.82	5.62[a]	0	94.38[b]	5.62[a]
		Female	85.81	13.27	0.92[b]	0	99.08[a]	0.92[b]
		SEM[5]	1.348	1.295	0.765	0	0.765	0.765
	Challenge	Male	71.88[b]	21.18[a]	6.94	0	93.06	6.94
	Challenge	Female	85.83[a]	13.83[b]	0.35	0	99.65	0.35
	Nonchallenge	Male	83.23[a]	12.47[b]	4.30	0	95.70	4.30
	Nonchallenge	Female	85.80[a]	12.72[b]	1.48	0	98.52	1.48
	SEM[6]		1.907	1.832	1.081	0	1.081	1.081
P-value								
	Diet		0.462	0.644	0.832	.	0.832	0.832
	Challenge		0.004	0.009	0.487	.	0.487	0.487
	Sex		<0.0001	0.056	<0.0001	.	<0.0001	<0.0001
	Diet × Challenge		0.129	0.311	0.389	.	0.389	0.389
	Diet × Sex		0.318	0.517	0.459	.	0.459	0.459
	Challenge × Sex		0.004	0.041	0.084	.	0.084	0.084
	Diet × Challenge × Sex		0.064	0.092	0.578	.	0.578	0.578

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.
[3]SEM, Standard error of mean for n = 32.
[4,5]SEM, Standard error of mean for n = 48.
[6]SEM, Standard error of mean for n = 24.

Table 11. Effects of dietary additive and cocci challenge on woody breast incidence of male and female broilers at 43 days of age.

Diet[1]	Challenge[2]	Sex	Score 0	Score 1	Score 2	Score 3	Score 0+1	Score 2+3
Control			69.90	14.06^{b}	9.17	6.88	83.96	16.04
Antibiotic			56.22	20.97^{a}	12.60	10.21	77.19	22.81
Probiotic			61.21	20.23^{a}	11.23	7.34	81.43	18.57
SEM[3]			2.303	2.031	1.845	1.682	2.258	2.258
	Challenge		58.59	18.96	12.43	10.02	77.55^{b}	22.45^{a}
	Nonchallenge		66.29	17.88	9.57	6.26	84.17^{a}	15.83^{b}
	SEM[4]		1.880	1.658	1.507	1.374	1.843	1.843
		Male	51.08^{b}	20.22	15.17^{a}	13.52^{a}	71.30^{b}	28.70^{a}
		Female	73.80^{a}	16.62	6.83^{b}	2.75^{b}	90.42^{a}	9.58^{b}
		SEM[5]	1.880	1.658	1.507	1.374	1.843	1.843
Control	Challenge		61.18^{b}	16.88	12.57	9.38	78.06	21.94
Control	Nonchallenge		78.61^{a}	11.25	5.76	4.38	89.86	10.14
Antibiotic	Challenge		54.65^{b}	21.81	14.10	9.44	76.46	23.54
Antibiotic	Nonchallenge		57.78^{b}	20.14	11.11	10.97	77.92	22.08
Probiotic	Challenge		59.93^{b}	18.19	10.63	11.25	78.13	21.88
Probiotic	Nonchallenge		62.48^{b}	22.26	11.84	3.42	84.74	15.26
SEM[6]			3.257	2.872	2.610	2.379	3.193	3.193
P-value								
	Diet		0.0003	0.036	0.419	0.321	0.321	0.107
	Challenge		0.005	0.648	0.184	0.056	0.013	0.013
	Sex		<0.0001	0.129	0.0002	<0.0001	<0.0001	<0.0001
	Diet × Challenge		0.040	0.244	0.312	0.138	0.275	0.275
	Diet × Sex		0.874	0.661	0.691	0.957	0.851	0.851
	Challenge × Sex		0.978	0.219	0.356	0.663	0.281	0.281
	Diet × Challenge × Sex[7]		0.542	0.302	0.730	0.355	0.567	0.567

[a,b]Means in a column not sharing a common superscript are different ($P < 0.05$).

[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).

[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.

[3]SEM, Standard error of mean for n = 32.

[4,5]SEM, Standard error of mean for n = 48.

[6]SEM, Standard error of mean for n = 16.

[7]Treatment means of treatment combinations were shown in Table 10.

incidence of normal breasts compared with that were fed control diet ($P = 0.040$; Table 11). Upon separating the means of all the treatment combinations (Table 12), it was observed that males than those fed antibiotic and probiotic diets exhibited a lower incidence of normal breasts than those fed the control diet ($P < 0.05$). In females, birds fed the antibiotic diet exhibited a lower incidence of normal breasts as compared to those fed the control diet ($P < 0.05$). Birds fed antibiotic and probiotic diets exhibited a higher incidence of WB that was scored 1 ($P = 0.036$; Table 11). Male birds exhibited a lower incidence of WB score 0 and 0 + 1 breast (for all $P < 0.0001$), but a higher incidence of WB score 2 and 2 + 3 ($P = 0.0002$ and $P < 0.0001$). The challenge resulted in a lower incidence of WB

Table 12. Effects of dietary additive and cocci challenge on woody breast incidence of male and female broilers at 43 days of age.

Diet[1]	Challenge[2]	Sex	Score 0	Score 1	Score 2	Score 3	Score 0+1	Score 2+3
Control	Challenge	Male	47.50^{c}	17.50^{bc}	20.00^{a}	15.00^{abc}	65.00^{e}	35.00^{a}
Control	Challenge	Female	74.86^{b}	16.25^{bc}	5.14^{cd}	3.75^{e}	91.11^{ab}	8.89^{de}
Control	Nonchallenge	Male	68.47^{b}	13.75^{bc}	9.03^{bcd}	8.75b^{cde}	82.22^{bc}	17.78^{cd}
Control	Nonchallenge	Female	88.75^{a}	8.75^{c}	2.50^{d}	0.00^{e}	97.50^{a}	2.50^{e}
Antibiotic	Challenge	Male	44.31^{c}	22.92^{ab}	19.03^{ab}	13.75^{abcd}	67.22^{de}	32.78^{ab}
Antibiotic	Challenge	Female	65.00^{b}	20.69^{ab}	9.17^{bcd}	5.14^{de}	85.69^{abc}	14.31^{cde}
Antibiotic	Nonchallenge	Male	47.36^{c}	20.28^{ab}	14.31^{abc}	18.06^{ab}	67.64^{de}	32.36^{ab}
Antibiotic	Nonchallenge	Female	68.19^{b}	20.00^{abc}	7.92^{cd}	3.89^{e}	88.19^{abc}	11.81^{cde}
Probiotic	Challenge	Male	50.00^{c}	17.50^{bc}	13.75^{abc}	18.75^{a}	67.50^{de}	32.50^{ab}
Probiotic	Challenge	Female	69.86^{b}	18.89^{abc}	7.50^{cd}	3.75^{e}	88.75^{abc}	11.25^{cde}
Probiotic	Nonchallenge	Male	48.85^{c}	29.38^{a}	14.93^{abc}	6.84^{cde}	78.23^{cd}	21.77^{bc}
Probiotic	Nonchallenge	Female	76.11^{ab}	15.14^{bc}	8.75^{bcd}	0.00^{e}	91.25^{ab}	8.75^{de}
SEM[3]			4.062	4.062	3.691	3.365	4.515	4.515
P-value								
Diet × Challenge × Sex			0.542	0.302	0.730	0.355	0.567	0.567

[a-e]Means in a column not sharing a common superscript are different ($P < 0.05$).

[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).

[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acerivulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.

[3]SEM, Standard error of mean for n = 8.

scored 0 + 1 and an increased incidence of WB scored 2 + 3 (for all $P = 0.013$; Table 11).

Correlation Between WB and Processing Yield

Spearman's correlation coefficients between WB score and processing yield were calculated. The relationship between WB score and breast muscle percentage was significant. The positive correlation between WB score and breast muscle percentage was found in every treatment group with the exception of challenged females fed the control or probiotic diet ($P = 0.112$ and $P = 0.343$, respectively) and the nonchallenged male birds fed the probiotic diet ($P = 0.677$). WB score was negatively associated with wings yield (relative weight) in nonchallenged male birds fed the control diet ($P = 0.01$), in nonchallenged male and female birds fed an antibiotic diet ($P = 0.001$ and $P = 0.003$, respectively), and in all birds fed the probiotic diet with the exception of nonchallenged male birds ($P = 0.003$, $P = 0.025$, and $P = 0.002$, respectively). WB score was negatively associated with drumsticks yield (relative weight) in nonchallenge male birds fed the control diet ($P = 0.01$), in all birds fed the antibiotic (for all $P < 0.05$), and in challenged male birds fed the probiotic diet ($P = 0.005$).

DISCUSSION

Recent studies have reported that nutritional strategies such as the use of feed additives could alleviate WB myopathy (Cruz et al., 2017; Bodle et al., 2018; Livingston et al., 2019). However, male and female commercial broilers are subject to complex rearing factors including diet management, diseases, and environmental conditions. However, the interactions between diet, diseases, and sex on WB development are still unknown. Thus, it was hypothesized that broiler diet (antibiotics and probiotics) and gut disease (coccidiosis) would affect the development of WB in male and female broilers.

Cocci Challenge

Cocci-challenged birds exhibited increased numbers of cocci Duo white and Jej red lesions on d 28 (Figure 1). Interestingly, birds in the cocci challenge group exhibited a 14% higher level of no-lesion cases as compared to nonchallenge groups on d 42 (Figure 2). This result suggests that the cocci challenge helped broilers to recover from the intestinal effects of coccidiosis eventually. A possible explanation for this might be that the shedding of cocci oocysts in the challenged group served as secondary antigens to evoke an immune response, thus protecting the intestinal epithelium of birds infected by *Eimeria* spp. (Chapman and Rayavarapu, 2007; Fetterer et al., 2015).

The cocci challenge not only induced gut lesions and the recovery but also caused inconsistent growth in broilers. A decline in BWG from d 14 to 29 (14-d postinfection was observed, but an increase in BWG from d 29 to 35 and from d 35 to 43 (Table 3) was observed. These results indicate that the cocci challenge positively affected growth performance during the later phases of growth after 15 d postchallenge. Similar results were reported by Wang et al. (2018), in which a cocci challenge increased FCR from d 15 to 40, but didn't further affect FCR after d 41. Limited research has been conducted on compensatory growth during the recovery phase after a cocci challenge. However, it is likely that recovery from the cocci infection and an associated increase in immunity level may have contributed to improved growth performance. WB incidence is highly related to an accelerated growth rate (Kawasaki et al., 2018), and the growth acceleration in the recovery phase after the cocci challenge in this trial may have contributed to the development of WB.

This study confirms that the cocci challenge increased WB incidence from d 29 to 43 (Tables 9–12).The *Eimeria* spp. infection caused by a cocci challenge has an impact on multiple health conditions in broilers including ceca microbiome diversity, nutrient digestion, amino acid and sugar metabolism, and immune reaction (Hong et al., 2006; Su et al., 2014; Macdonald et al., 2017; Miska and Fetterer, 2017). Primarily, *E. acervulina* infection affects the protein metabolism of challenged birds and decreases methionine absorption (Sharma and Fernando, 1975; Ruff et al., 1976). Broilers who suffer from *Eimeria* spp. infection may have intestinal lesions and an exacerbated inflammatory response, which causes oxidative stress to the birds (Sepp et al., 2012). Further studies are needed to determine the effects of a cocci challenge on other related variables such as gut microbiota composition, stress-related gene expression, serum immunoglobulin levels, and nutrient metabolism-related enzyme activities to understand the possible factors which might contribute to the development of WB.

Diet

On d 43, in nonchallenged groups across sex, birds fed the probiotic or antibiotic diets exhibited a decreased incidence of normal breast quality (score 0) (Tables 11 and 12). This finding was unexpected and suggests that *Bacillus subtilis* cannot alleviate WB in birds without a cocci infection. The benefits of probiotics include modulation of the number of pathogenic and beneficial microorganisms in the gastrointestinal tract. Subsequently, the growth and performance of challenged broilers is improved without the exhibition of disease symptoms. The oxidative status and stress response of these birds is likewise mitigated (Dalloul et al., 2003; Lee et al., 2010; Hossain et al., 2012; Giannenas et al., 2014; Wang et al., 2018). In terms of growth performance, the result of this study is contrary to previous findings that have suggested that probiotics promote broiler growth. In the current study it was found that *Bacillus subtilis* did not affect growth performance but increased WB incidence.

What is surprising is that the WB condition was expected to relate to an increase in growth performance. However, these results indicated that *Bacillus subtilis* supplementation led to an increase in WB incidence without affecting BW, BWG, and GR (Tables 3 and 6). Intestinal microbiota impacts the host in terms of its nutritional, physiological, and immunological statuses (Diaz Carrasco et al., 2019). The probable reason might be that the probiotic feed additive altered the gut microbiota, which changed the diversity or composition of the gut microbiota, which led to changes in oxidative stress level or the metabolism of nutrients, thus increasing the incidence of WB development (Zhang et al., 2021b). However, more research is needed to determine the mechanism behind the administration of probiotics and the development of WB. Studies would need to be carried out through market age (56 or 63 d of post hatch) to fully determine the impact of probiotic supplementation on WB incidence of broilers.

We found that birds fed the antibiotic diet exhibited an increased growth rate during the earlier age phase from d 0 to 14 (Table 6). Within the nonchallenge group, birds fed the antibiotic diet also exhibited a higher WB incidence as compared to birds fed the control diet (Tables 11 and 12). Subtherapeutic levels of antibiotic growth promoters (**AGPs**) have a tremendous impact on the growth of farm animals. The proposed mechanisms of AGPs' growth-promoting function are 1) reducing microbial density in the gastrointestinal tract so that preserving nutrient availability in the host, 2) promoting a favorable gut microbial homeostasis, 3) reducing the metabolism of pathogenic bacteria, and 4) improving nutrient absorption via a thinner gut epithelium (Dibner and Richards, 2005; Miles et al., 2006). In the current trial, the antibiotic diet increased BW, BWG, GR, and the antibiotic diet also increased incidence of WB. Rapid growth has been reported as the main contributor to the production of WB. Therefore, the increased BW, BWG, GR, or changed microbial homeostasis in response to the antibiotic treatment may have contributed to WB development.

Sex

Male birds exhibited a higher BW, BWG, and GR throughout all the growth phases examined and WB incidence was higher in males than in females (Tables 3 and 6). These results are in agreement with recent studies, which indicated that male broilers exhibited a higher live weight (3,492 vs. 2845 g) and a higher WB incidence (16.3 vs. 8.0 %) than female broilers (Trocino et al., 2015). In this study, male broilers also exhibited higher processing yields (relative weight) for breast muscle and drumsticks. Therefore, inadequate breast muscle vascularization is a potential key factor in the occurrence of WB (Zambonelli et al., 2016). Furthermore, male broilers who rapidly accumulate breast muscle during growth are more likely to have inadequate vascularization. Genotype, hormone, and behavior differences between male and female broilers may also relate to their differences in WB development.

Processing Yield and WB

WB score was positively related to breast muscle percentage (yield) 9 of 12 treatment combinations (Table 13). These results are consistent with recent research in which it was reported breast weight was positively related to WB incidence (Zhang et al., 2021a). Likewise, in other studies it was found that larger and heavier breasts have a higher severity of WB lesions

Table 13. Correlation of woody breast score and processing yield (relative weight/%).

Diet	Challenge	Sex		Carcass	Breast	Wing	Drumstick	Fat pad	Thigh	Tender
Control	Challenge	Male	Coefficient	0.285	0.324	−0.01	−0.26	−0.098	−0.199	−0.063
			P-value	0.075	0.041	0.951	0.106	0.547	0.219	0.7
Control	Challenge	Female	Coefficient	0.382	0.255	−0.098	−0.15	−0.11	−0.112	0.163
			P-value	0.015	0.112	0.548	0.357	0.499	0.494	0.314
Control	Nonchallenge	Male	Coefficient	0.3	0.509	−0.402	−0.403	0.296	−0.199	−0.287
			P-value	0.06	0.001	0.01	0.01	0.064	0.218	0.076
Control	Nonchallenge	Female	Coefficient	0.231	0.376	0.035	−0.223	−0.052	−0.149	−0.215
			P-value	0.152	0.017	0.83	0.166	0.753	0.359	0.183
Antibiotic	Challenge	Male	Coefficient	0.256	0.506	−0.196	−0.561	−0.127	−0.119	−0.165
			P-value	0.112	0.001	0.225	0.0002	0.434	0.467	0.309
Antibiotic	Challenge	Female	Coefficient	0.061	0.439	−0.137	−0.36	0.005	−0.399	0.414
			P-value	0.711	0.005	0.4	0.023	0.976	0.011	0.008
Antibiotic	Nonchallenge	Male	Coefficient	0.286	0.593	−0.51	−0.387	0.05	−0.43	0.066
			P-value	0.074	<0.0001	0.001	0.014	0.762	0.006	0.686
Antibiotic	Nonchallenge	Female	Coefficient	0.34	0.625	−0.466	−0.311	−0.206	−0.1	−0.021
			P-value	0.032	<0.0001	0.003	0.051	0.202	0.54	0.9
Probiotic	Challenge	Male	Coefficient	0.513	0.432	−0.464	−0.439	−0.391	−0.079	−0.23
			P-value	0.001	0.005	0.003	0.005	0.013	0.627	0.153
Probiotic	Challenge	Female	Coefficient	0.237	0.156	−0.358	−0.167	0.049	−0.045	−0.027
			P-value	0.142	0.343	0.025	0.309	0.765	0.787	0.873
Probiotic	Nonchallenge	Male	Coefficient	0.087	0.068	−0.273	−0.172	0.026	−0.335	0.198
			P-value	0.595	0.677	0.089	0.288	0.872	0.034	0.221
Probiotic	Nonchallenge	Female	Coefficient	0.305	0.488	−0.487	−0.588	0.063	−0.041	0.267
			P-value	0.056	0.001	0.002	<0.0001	0.701	0.801	0.096

(Cruz et al., 2017; Kuttappan et al., 2017; Radaelli et al., 2017). The relationship between breast meat weight and WB score may be due to the fact that larger breast muscles may suffer more from oxygen and nutrition shortage, which subsequently causes muscle necrosis (Hoving-Bolink et al., 2000).

Negative correlations existed ($P < 0.05$) between WB score and wing drumstick yield (percentage). This suggests that broilers exhibiting WB have less muscle development in wings and legs, whereas the breast muscle is better developed due to its more rapid growth. A hypothetical basis for this is that birds with WB may have impaired movement (Norring et al., 2019), in association with weaker wing and drumstick function.

In conclusion, antibiotic additives and *Eimeria* spp. infection promoted the development of WB in broilers via interruption of their growth rate. Probiotic supplementation increased the WB incidence of male broilers without affecting their BW and GR. Results also confirm that incidence of WB increases in percentage as the broiler gets older and that greater growth rate also leads to greater incidence of WB.

ACKNOWLEDGMENTS

This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station. This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession numbers of MIS-329250/NE-1442 and MIS-322370.

DISCLOSURES

The authors have declared no conflict of interest.

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